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The epidemiology of zoonoses in slaughterhouse workers in western Kenya

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“The doctor of the future will give no medicine, but will educate his patients in the care of the human frame, in diet, and in the cause and prevention of disease.”

Thomas Edison

Declaration

The data presented in this thesis are my own. There are components of the data collection and sample analysis that were conducted by others. These are outlined below. In all circumstances I performed the analysis and interpretation of the data.

The field data collection was conducted by a team of medical, veterinary and laboratory personnel including myself.

Human sampling was conducted by Fred Amanya, Daniel Cheruiyot and Lorren Alumasa.

Animal sampling was conducted by James Akoko, Lazarus Omoto, Will de Glanville, Lian Thomas and myself.

Human parasitological testing was performed by Hannah Kariuki and John Mwaniki.

Serological testing was conducted by Alice Kiyong'a and Velma Kivali together with myself at the International Livestock Research Institute, Nairobi, Kenya.

This work has not been submitted for any other degree or professional qualification.

Signed: 

Date: 21st May 2015

Abstract

Slaughterhouses are places where animals are slaughtered for food. In developing countries a lack of appropriate facilities and limited resources mean the slaughter industry is poorly regulated. Poor hygiene practices in slaughterhouses can result in the transmission of diseases from animals to people called zoonoses. Slaughterhouse workers are generally considered at increased risk of being exposed to such diseases due to their close contact with animals and animal products.

The aims of this study were: to assess the current conditions in slaughterhouses and the knowledge, attitudes and practices of workers in ruminant and pig slaughterhouses in western Kenya; to determine the exposure of slaughterhouse workers to different zoonotic pathogens; to investigate the risk factors associated with exposure to these pathogens and to quantify the risk of zoonotic disease exposure for slaughterhouse workers compared to the general population.

Slaughterhouses in western Kenya were visited between May 2011 and October 2012. Seven hundred and thirty-eight workers were recruited from 142 slaughterhouses. Overall, the slaughterhouses lacked facilities, with 65% (95% CI 63–67%) of slaughterhouses having a roof, cement floor and solid sides, 60% (95% CI 57–62%) had a toilet and 20% (95% CI 18–22%) hand-washing facilities. Less than half of workers 32% (95% CI 29–34%) wore personal protective clothing. Antemortem inspection was practiced at 7% (95% CI 6–8%) of slaughterhouses and 18% (95% CI 16–19%) of workers reported slaughtering sick animals.

Slaughterhouse workers were screened for five zoonotic diseases. The unadjusted seroprevalence of the zoonotic diseases were: brucellosis 0.1% (95% CI 0.007–

0.8%); leptospirosis 13.4% (95% CI 11.1–16.1%); Q fever 4.5% (95% CI 3.2–6.2%); Rift Valley fever (RVF) 1.2% (95% CI 0.6–2.3%); taeniasis 1.8% (95% CI 1.0–3.0%); and cysticercosis 2.6% (95% CI 1.7–4.0%).

Risk factors for leptospirosis and Q fever were examined by multivariable logistic regression. Risk factors associated with leptospirosis seropositivity included: having wounds (OR 2.7; 95% CI 1.4–5.3); smoking at work (OR 1.8; 95% CI 1.1–3.0); eating at work (OR 2.1; 95% CI 1.2–3.6); and cleaning the intestines (OR 3.8; 95% CI 1.8–8.2). Protective factors were: working at a slaughterhouse where antemortem inspection was performed (OR 0.6; 95% CI 0.4–0.9). The risk factors significantly associated with Q fever seropositivity included: being intoxicated at work (OR 3.2; 95% CI 1.1–9.4).

The odds ratio for leptospirosis seropositivity in slaughterhouse workers was determined to be 2.3 (95% CI 1.6–3.4) times that of the community. For Q fever the odds ratio for seropositivity in slaughterhouse workers was 1.9 (95% CI 1.0–3.8) times that of the community.

This is the first report of a range of zoonotic pathogens in slaughterhouse workers in Kenya. This study indicated the potential risk factors for zoonotic disease exposure in slaughterhouses. The current working conditions in slaughterhouses in western Kenya are far below the recommended standard. Improvements need to be made to facilities and practices in all slaughterhouses. Training is recommended to improve awareness for workers, managers and inspectors of the risks of zoonotic disease exposure and methods to reduce it.

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The creation of a thesis can seem at times a dark and lonely road – usually at 3am sitting alone amongst the papers and empty coffee mugs. In fact I have had an abundance of support. Many people have given advice, sent R code when I didn't have a clue, made tea or more often poured a glass of wine. I am fortunate to have many remarkable friends, colleagues and advisors that made this possible.

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List of Abbreviations

Ab	Antibody
AIC	Akaike's second-order information criterion
BD	Becton, Dickinson and Company
CI	Confidence interval
CT	Computerised tomography
DLPO	Divisional livestock production office
EDTA	Ethylenediaminetetraacetic acid
EITB	Enzyme-linked immunoelectrotransfer blot
ELISA	Enzyme-linked immunosorbent assay
FAO	Food and Agriculture Organization of the United Nations
fpc	Finite population correction
GIS	Geographical information systems
GPS	Global Positioning System
HDSS	Human and Demographic Surveillance System
HIV	Human immunodeficiency virus
Ig	Immunoglobulin
IHA	Indirect haemagglutination assay
ILRI	International Livestock Research Institute
KEMRI	Kenya Medical Research Institute
MAT	Microscopic agglutination test
MCA	Multiple correspondence analysis
MOR	Median odds ratio
MRC	Medical Research Council
MRI	Magnetic resonance imaging
MUAC	Mid-upper-arm circumference
OD	Optical density
OIE	World Organisation for Animal Health

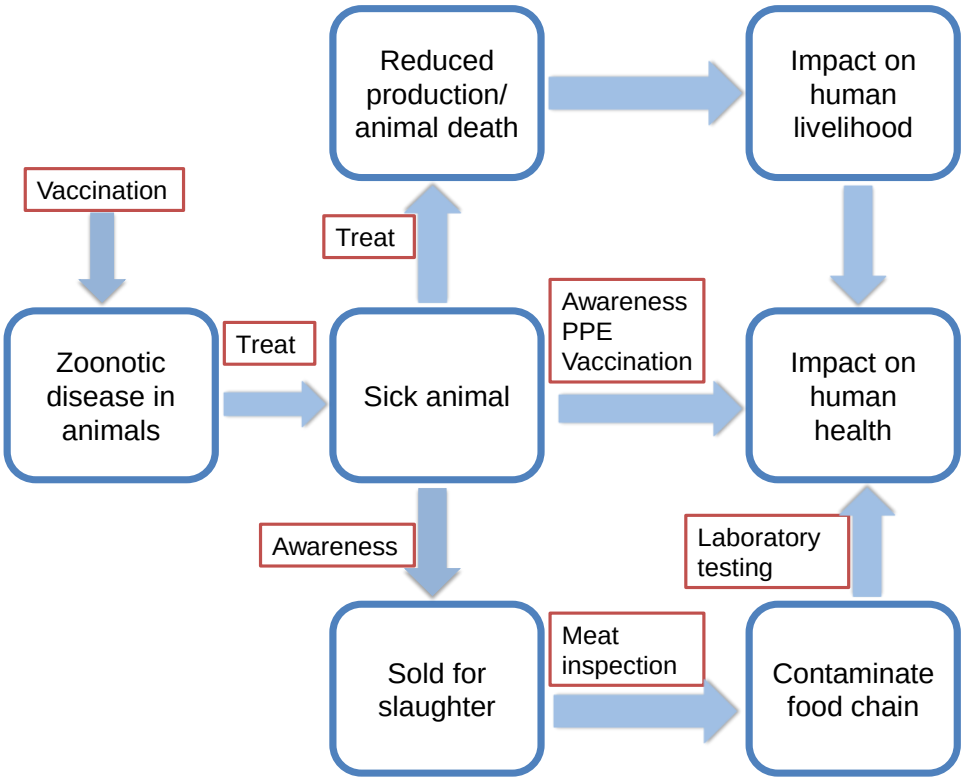
OR	Odds ratio
PAZ	People, Animals and their Zoonoses
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PCV	Packed cell volume
PDA	Personal digital assistant
PI	Percentage inhibition
PPE	Personal protective equipment
RBT	Rose Bengal test
rpm	Revolutions per minute
RVF	Rift Valley fever
SE	Standard error
Se	Sensitivity
Sp	Specificity
SSA	Sub-Saharan Africa
USA	United States of America
VIFs	Variance inflation factors
VPH	Veterinary public health
WHO	World Health Organization

Chapter 1

Introduction

1.1 Motivation

Zoonotic diseases are those that are transmitted between animals and people (WHO, 2006). The burden of zoonotic disease falls disproportionately on poor people in rural areas who live in close contact with their animals (Maudlin et al., 2009). Livestock serve many functions for people in rural areas including: food source, traction/transport, manure, dowry, and financial security (Muma et al., 2014). Zoonotic diseases impact both human health and also livelihoods as disease in animals can result in reduced livestock productivity (Figure 1.1) (Maudlin et al., 2009).



*The red boxes indicate where interventions may reduce the impact of the disease to people/animals.
PPE – personal protective equipment

Figure 1.1 The effects of zoonotic disease.

(Adapted from (FAO, 2002).

The surveillance and control of zoonotic diseases requires a "One Health" approach, involving human and animal health disciplines (WHO, 2006). However, lack of funding and inadequate coordination has weakened animal health and veterinary departments in most developing countries. This situation has resulted in a lack of control of endemic zoonoses, increasing occurrences of foodborne disease and the emergence of new diseases (FAO, 2002). These veterinary public health (VPH) issues are likely to be exacerbated in future by globalisation, increasing population, urbanisation, climate change, changing agricultural practices, and agricultural intensification (WHO, 2002).

Improvements in VPH systems in developing countries will require a focus on education of stakeholders, regulation and legislation, improved facilities, new technologies, surveillance, and communication (WHO, 2002). In addition, interdisciplinary research is necessary to understand the epidemiology of zoonoses in different environments, to perform appropriate risk analyses, and to develop control measures (WHO, 2002).

In 2006, a joint meeting between the World Health Organization (WHO), the Food and Agriculture Organization of the United Nations (FAO) and the World Organisation for Animal Health (OIE) made recommendations on methods to control zoonotic disease for poverty alleviation in developing countries (WHO, 2006). One of the epidemiological research methods proposed by the meeting was the investigation of risk factors for zoonoses in high risk groups for the purpose of developing targeted control interventions. One such high risk group is slaughterhouse workers (McEwen, 1987). In areas where animal disease surveillance systems are

poor, it has been suggested that slaughterhouse workers may act as sentinels for monitoring zoonotic diseases (Rabinowitz et al., 2009).

The People, Animals and their Zoonoses (PAZ) project was established in 2010 to investigate the epidemiology of zoonoses in livestock and non livestock keeping homesteads in rural western Kenya (Doble and Fevre, 2010). The study of zoonotic diseases in slaughterhouse workers is an off-shoot of the PAZ project. It came about because of a request by slaughterhouse workers within the PAZ study area for information regarding their zoonotic disease risks. These slaughterhouse workers presented a unique opportunity to conduct a demand-led project with the support of the community.

The project proposal and protocols were developed in 2011 during the first year of the author's PhD. The field data collection and initial laboratory testing was performed at the International Livestock Research Institute (ILRI) laboratory in Busia, Kenya in 2012. The majority of the serological testing was conducted at the ILRI laboratory in Nairobi, Kenya in 2013. The data analysis and write up was completed in 2014.

1.2 Aims

The project aim was to understand the epidemiology of zoonoses in slaughterhouse workers in rural western Kenya. The main hypotheses of this study are that:

1. Slaughterhouses in western Kenya have inadequate infrastructure, sanitation and hygiene practices;

2. The current situation in slaughterhouses in western Kenya contributes to zoonotic disease risk in slaughterhouse workers;
3. Slaughterhouse workers are more exposed to zoonotic disease than other members of the community.

The specific aims of this thesis are to:

1. describe the current standards and practices in slaughterhouse workers in western Kenya;
2. to measure the seroprevalence of zoonotic pathogens in slaughterhouse workers;
3. identify the risk factors for zoonotic disease exposure in slaughterhouse workers;
4. compare the prevalence of zoonotic disease in slaughterhouse workers and the community.

1.3 Thesis outline

The overall aim of this thesis is to establish the exposure of slaughterhouse workers to zoonotic disease and the risk factors associated with exposure.

Chapter 1 is a review of the literature regarding slaughterhouses and the specific zoonotic diseases that will be the focus of this study.

Chapter 2 describes the design of the study which was conducted in western Kenya between May 2011 and October 2012.

Chapter 3 describes the current standards and practices in slaughterhouses and amongst slaughterhouse workers in western Kenya and compares these practices between different slaughterhouse types.

Chapter 4 discusses the diagnostic tests used to determine the exposure of slaughterhouse workers to zoonotic disease and reports the prevalence of zoonotic disease in the different slaughterhouse types.

Chapter 5 identifies the risk factors for leptospirosis and Q fever seropositivity among slaughterhouse workers in western Kenya.

Chapter 6 identifies the risk factors for leptospirosis and Q fever seropositivity in a community-based sample of the population of western Kenya.

Chapter 7 explores the difference in the seropositivity to zoonotic disease between slaughterhouse workers and members of the community.

Chapter 8 summarises the main findings of the thesis and makes recommendations for future research areas and potential areas where interventions for control may be effective.

1.4 Slaughterhouses

A slaughterhouse, also called an abattoir, is defined as a place where animals are slaughtered for food (Stevenson, 2013). The development of the slaughter industry varies between countries due to cultural differences, the types of animals slaughtered and wealth (Long, 1990). In developed countries such as the USA or United Kingdom slaughter facilities are centralised, large-scale, industrialised, and are predominantly meat packing plants where meat is packed ready for distribution

(Broadway, 2002, Broadway and Ward, 1990). This growth in the industry occurred through urbanisation, improved transport and refrigeration, in addition to regulations for public health (Fitzgerald, 2010). In developing countries there are three types of slaughter facilities (Clottey, 1985).

- The larger cities or towns often have government or commercially-owned modern slaughterhouses that are well designed and equipped with industrial meat processing facilities. These slaughterhouses focus on the commercial or export markets.

- In smaller towns local authorities own and manage the slaughterhouses and slaughterslabs[1]. These facilities are rented to butchers to slaughter animals for the local market.

- In villages and rural areas slaughter facilities are privately owned and unregulated.

This variation in standards is largely due to inadequate infrastructure to regulate the trade particularly in rural areas. In addition there is often a deficit of suitable and/or affordable equipment for the processing and transportation of meat. Overall there is a lack of incentive to improve conditions which is the result of a poor understanding of the risks of foodborne disease (Mann, 1984, FAO, 2010).

[1] Slaughterslab is a term used to describe crudely equipped low throughput slaughter facilities in rural areas. For the purposes of this thesis the term slaughterhouse will refer to all slaughter facilities.

1.4.1 Risks of an unregulated meat industry

The objective of regulation within the slaughter industry is to reduce the transmission of zoonoses (Mann, 1984). There are a number of points along the slaughter chain for potential hygiene infringements leading to contamination of meat or spread of disease (Figure 1.2). In most situations the failure of slaughterhouses to maintain meat hygiene occurs due to inadequate infrastructure, poor hygiene, lack of ante and post mortem inspection, and inadequate training (Figure 1.3) (FAO, 2010, Herenda, 1994).

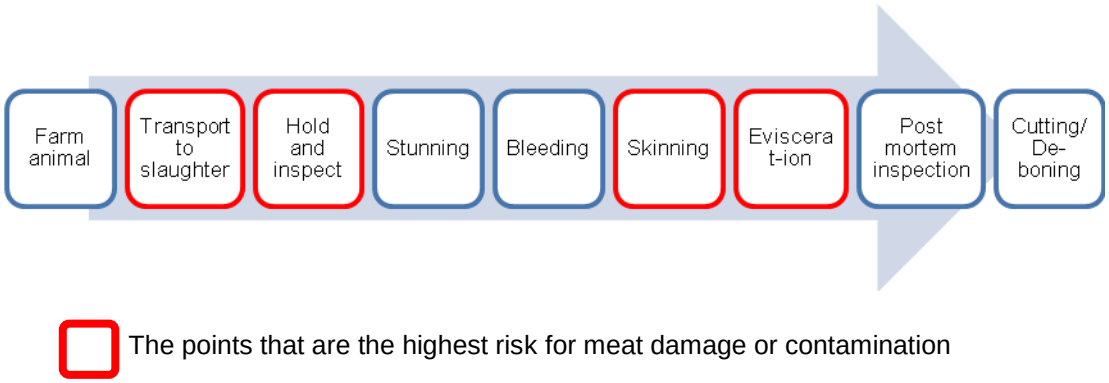
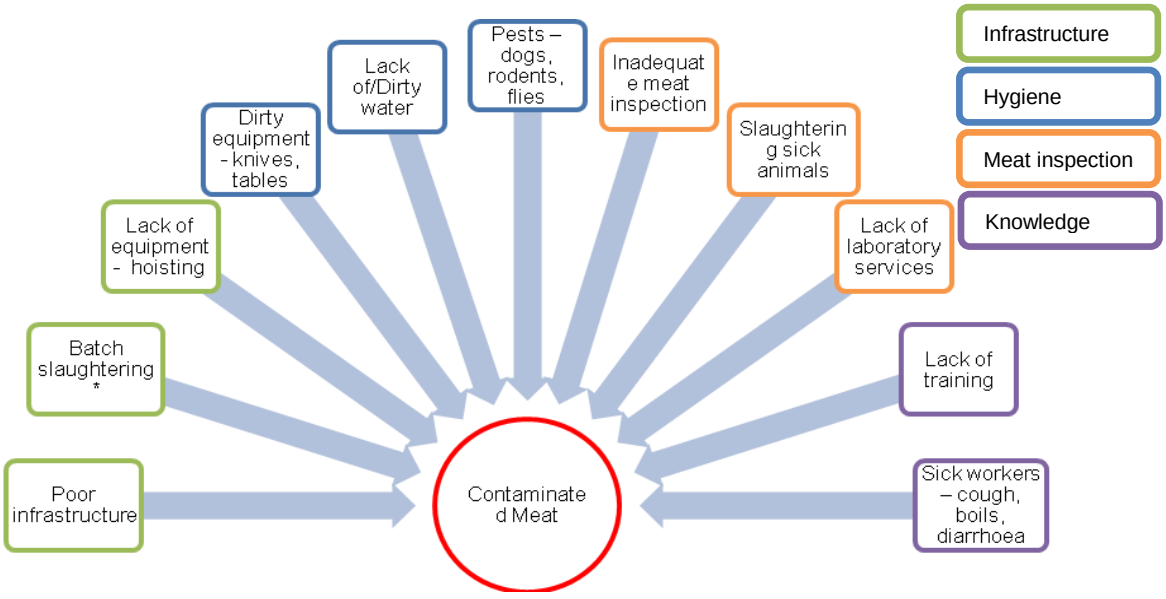


Figure 1.2 The slaughterhouse process

Adapted from *Manual on meat inspection in developing countries* (Herenda, 1994)



* Batch slaughtering is where all processes (bleeding, skinning, evisceration and cutting) are performed in the same spot (FAO, 2010)

Figure 1.3 Factors contributing to meat contamination.

1.4.2 Infrastructural requirements

The FAO published international guidelines for minimum building requirements in slaughterhouses (FAO, 2004, Codex Alimentarius Commission, 2005). The FAO guidelines take into account the availability of resources in different regions. The guidelines recommend that buildings should be situated away from residential areas with adequate land for expansion. Roofing is recommended and the floor must be hard, smooth, and impervious. There should be separate areas for stunning, bleeding, scalding, and skinning before evisceration. Hoisting equipment is essential. There should be animal holding facilities, a source of potable water, toilets, and a pit for disposal of carcasses and condemned meat.

1.4.3 Personal hygiene requirements

Workers can be a source of meat contamination through poor personal hygiene (FAO, 2004). The FAO guidelines suggest the following steps to reduce meat contamination from personnel:

- clean coveralls and waterproof boots that are only worn in the slaughterhouse;
- workers should abstain from work if coughing, sneezing, or have gastrointestinal illness;
- cuts or abrasions should be covered with waterproof tape;
- workers should wash hands with soap and warm water when starting and finishing work, after using the toilet, after coughing, sneezing, or touching the face.

1.4.4 Meat inspection

Meat inspection is important to reduce the spread of disease. Antemortem inspection prevents diseased animals entering the slaughterhouse and hence the food chain (Herenda, 1994). Disease monitoring at the slaughterhouse can be reported back to source farms. This reporting can lead to animal disease control at the farm level and reduce the occurrence of animal pathogens in the surrounding region (Mann, 1984). In this way, antemortem screening for tuberculosis in cattle has formed an essential part of eradication programmes in many countries (Palmer and Waters, 2011).

Antemortem inspection is particularly important in developing countries where there may be a higher proportion of sick animals at slaughter. Producers may sell sick animals to reduce losses (Brown et al., 2011). Animals with brucellosis, for example, may be sold due to previous abortion or infertility. These animals are likely to shed large amounts of the infectious organism posing a risk to workers and consumers (Nabukenya et al., 2013). Postmortem inspection of the carcass is important to identify lesions. This inspection is done using gross examination combined with laboratory support to determine the fitness of the carcass for human consumption (FAO, 2010). Postmortem inspection should be risk based (Codex Alimentarius Commission, 2005):

- according to knowledge of the animal disease problems in the region;
- allowing additional examination of carcasses where findings suggest the presence of disease;
- reducing cross contamination through proper handling of the carcass.

1.4.5 Training

The meat industry employs unskilled labour with a high turnover which makes specialisation difficult (Fitzgerald, 2010). Training of slaughterhouse workers in personal hygiene practices has been shown to significantly reduce carcass contamination (Wamalwa et al., 2012). Education regarding the risks of zoonotic disease can reduce exposure to disease (Campagnolo et al., 2000, Nabukenya et al., 2013). However in developing countries slaughtering is done by butchers with their own team in rented slaughter facilities. The slaughterhouses are not staffed with regular personnel, which makes consistent and formal training difficult (FAO, 2010).

1.4.6 Slaughterhouse worker health

Slaughterhouse workers are considered a high risk group for work-related injuries and occupational exposure to disease (Fitzgerald, 2010). Slaughterhouse workers are at particularly high risk of lacerations to the arms and hands through the use of hand-held tools (Cai et al., 2005, Pedersen et al., 2010, Burridge et al., 1997). They are also at risk of contracting zoonoses due to their intimate contact with animals, animal products, and excreta (Beheshti et al., 2010, Dorjee et al., 2011). Transmission of these diseases occurs through the regular routes such as inhalation, instillation in wounds or ingestion (Taylor et al., 2001). Exposure is exacerbated under slaughterhouse conditions by opening the carcass, being splashed with body fluids, poor hygiene practices, and working with uncovered wounds (Mann, 1984). Risk behaviours that appear most frequently to be associated with zoonotic disease exposure are low levels of education, role in the slaughterhouse, lack of personal protective equipment, smoking, and eating at work (Brown et al., 2011, Gilbert et al., 2012, Wilson et al., 2010). Zoonotic diseases that have been reported in

slaughterhouse workers, worldwide, include anthrax, brucellosis, leptospirosis, Rift Valley fever, orf virus, dermatophytosis, and Q fever (Beheshti et al., 2010, Brown et al., 2011, Wilson et al., 2010, Ray et al., 2009, Peck and Fitzgerald, 2007, Maslen, 2000, Nougairede et al., 2013, Abu-Elyazeed et al., 1996).

Four zoonotic diseases were identified to commonly affect slaughterhouse workers and are the focus of this study – brucellosis, leptospirosis, Q fever, and Rift Valley fever. Table 1.1 shows a selection of published reports of these diseases in slaughterhouse workers. Two additional diseases were investigated: taeniasis and cysticercosis. The reason for including these diseases is discussed later in the chapter.

1.5 Epidemiology of zoonotic disease

Little is known about the prevalence of zoonotic diseases in sub-Saharan Africa due to the lack of surveillance, misdiagnosis, and underreporting (McDermott and Arimi, 2002, Abela-Ridder et al., 2010, Maudlin et al., 2009). The generalised presentation in people of zoonotic diseases such as brucellosis, leptospirosis and Q fever result in misdiagnoses as other febrile illnesses such as malaria, typhoid, tuberculosis, cancer or HIV-related illness (Mantur et al., 2006, Dames et al., 2005, McDermott and Arimi, 2002, Abela-Ridder et al., 2010, Crump et al., 2013). Unfortunately diagnostic tests for zoonotic diseases are often not available or have variable performance in endemic situations (Crump et al., 2013, Maudlin et al., 2009). Diagnosis of febrile illnesses is further complicated by co-infections with multiple pathogens or previous infections (Ari et al., 2011).

Disease	Study type	Country	Animals slaughtered	Prevalence (people)	Risk factors	Year of study	Reference
Brucellosis	Prevalence	Pakistan	Cattle, Goats, Sheep, Camels, Buffaloes	21.7%	Age Assistance at animal birth Consuming raw milk Handling sheep	2008	Mukhtar, 2010
	Prevalence	Uganda - 2 regions	Cattle, Goats, Sheep	10% 7%	Lack of protective gear Working over 5 years	2007	Nabukenya et al., 2013
	Prevalence	Iran	Cattle Sheep	9.8%	Longer duration of work Contact with sheep Traditional slaughterhouse	2009	Nikokar et al., 2011
Leptospirosis	Outbreak	USA	Pigs	8%	Smoking Drinking at work Washing hands (protective)	1998	Campagnolo et al., 2000
	Prevalence	Singapore	Pigs	28.7%	Cleaning intestines	1987	Chan et al., 1987
	Prevalence	New Zealand	Sheep Deer Cattle	12-31% 17-19% 5%	Role/position in slaughterhouse	2009	Dreyfus et al., 2014
Q fever	Outbreak	Scotland	Sheep	41.9%	Work position	2006	Wilson et al., 2010
	Prevalence	Brazil	Cattle	29%	Work position	1972	Riemann et al., 1975
Rift Valley fever	Outbreak	Egypt	Cattle	2%	Cutting animals' throats Handling animal parts	1993	Abu-Elyazeed et al., 1996

Table 1.1 Published reports of zoonoses in slaughterhouse workers

1.5.1 Brucellosis

Brucellosis is considered to be one of the most widespread and most common zoonoses worldwide (Pappas et al., 2006). *Brucella abortus* from cattle is the most widespread species and *B. melitensis*, primarily from sheep and goats, causes the most cases of human disease (Corbel, 1997). There have been reports of both *B. abortus* and *B. melitensis* in Kenya (McDermott and Arimi, 2002, Corbel, 1997, Muendo et al., 2012).

Transmission of brucellosis to people is primarily through consumption of unpasteurised milk products from an infected animal but can also occur through instillation of bacteria into broken skin or inhalation of the organism (Pappas et al., 2005, Beheshti et al., 2010). The latter transmission events occur through contact with body fluids or excreta such as urine, faeces, blood, vaginal secretions, gravid uteri, or abortion material (Beheshti et al., 2010, Ali et al., 2013). Slaughterhouse workers are occupationally exposed to brucellosis because of their contact with body fluids of infected animals during evisceration or cleaning (Beheshti et al., 2010, Ali et al., 2013). Slaughterhouse workers have been demonstrated to be a high risk group for brucellosis in a number of studies with prevalence more than twice that of the general population (Ali et al., 2013, Nikokar et al., 2011, Swai and Schoonman, 2009, Abo-Shehada et al., 1996, Bikas et al., 2003).

Human brucellosis is a clinically non-specific illness that invariably presents with fever (Pappas et al., 2005). Clinical signs include headache, joint pain, back pain, sweating, lethargy, lymphadenopathy, splenomegaly, hepatomegaly, and epididymoorchitis (Pappas et al., 2005, Ali et al., 2013). In order to definitively

diagnose brucellosis, the organism must be isolated from blood or tissue (Pappas et al., 2005). Traditionally serological diagnosis has been made by serum agglutination but enzyme-linked immunosorbent assays (ELISAs) have improved sensitivity and specificity (Mantur et al., 2006). Lateral flow assays have been shown to be effective at diagnosing brucellosis at different stages of infection and are convenient field tests (Irmak et al., 2004). The Rose Bengal plate agglutination test (RBT) has also been shown to be a highly specific and sensitive for diagnosis of brucellosis (Cernyseva et al., 1977).

1.5.2 Leptospirosis

Leptospirosis is a zoonotic disease with worldwide distribution (Abela-Ridder et al., 2010). There are over 200 serovars of the pathogenic *Leptospira* and domestic animals are maintenance hosts for a number of pathogenic serovars including: cattle (hardjo, pomona, grippotyphosa); pigs (pomona, tarassovi, bratislava); and sheep (hardjo and pomona) (Levett, 2001). There is extremely limited published material regarding the prevalence of human leptospirosis in Kenya. The first human cases were reported in 1977 (de Geus et al., 1977) and in 2011 a study investigating acute febrile illnesses in northern Kenya reported cases of leptospirosis (Ari et al., 2011). A number of serovars have been reported in rodents in a recent study in Kenya, highlighting the potential public health risk posed by this zoonosis (Halliday et al., 2013).

Leptospires are maintained in the kidneys of the host animal and excreted in urine (Levett, 2001, Monahan et al., 2009). Human infections result from exposure through broken skin or mucosal surfaces to the organism in urine from an infected

animal or contaminated water or soil (Waitkins, 1986, Campagnolo et al., 2000). Faine et al (1999) described three epidemiological situations that promote the transmission of leptospirosis to people (Figure 1.4):

1. farming in temperate climates where transmission events occur predominantly from infected domestic animals – cattle and pigs
2. tropical wet areas with a range of animal reservoirs – rodent, cattle, pigs, and dogs
3. urban situations where rodents are the predominant reservoir

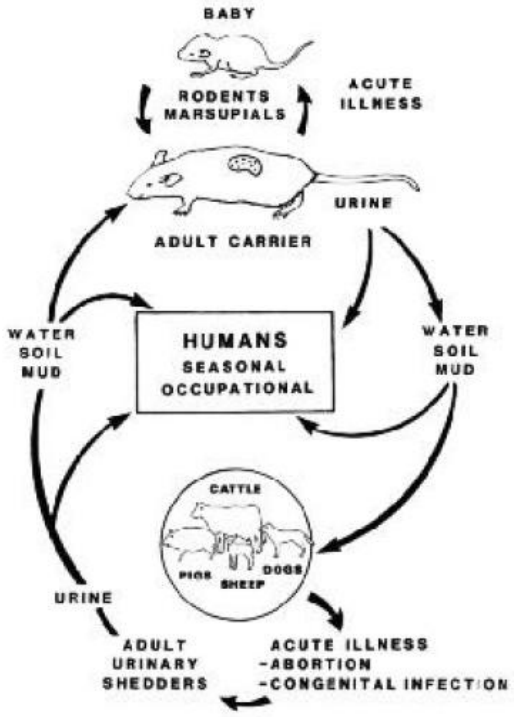
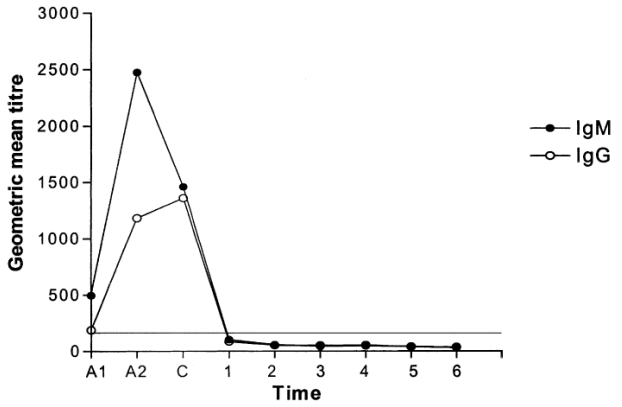


Figure 1.4 Transmission cycles of leptospires (Faine, 1999)

Farmers, veterinarians, slaughterhouse workers, rodent control officers and sewer workers are all considered to be occupationally exposed to *Leptospira* spp. (Campagnolo et al., 2000, Alston and Brown, 1935, Demers et al., 1985). Slaughterhouse workers are considered to be at high risk and have been shown to

have a seropositivity rate twice that of other non risk occupations in studies conducted in Singapore, Tanzania and India (Chan et al., 1987, Schoonman and Swai, 2009, Sharma et al., 2006). Other risk factors include recreational water sports such as swimming (Jackson et al., 1993, Evans, 2000).

The majority of human infections are subclinical or mild. Persons with leptospirosis will develop fever, headache, muscle pain, anorexia, nausea, vomiting, abdominal pain, rash, conjunctivitis, and hepatitis (Terry et al., 2000, Farr, 1995, Waitkins, 1986). A small number of patients will develop Weil's disease with jaundice, renal failure and haemorrhage (Bharti et al., 2003). The microscopic agglutination test (MAT) is currently the gold standard for sero-diagnosis of leptospirosis but is complex and requires experienced operators (Levett, 2001). Alternative methods include the indirect haemagglutination assay (IHA) which has variable performance; and ELISAs which are generally recommended as a screening tool for suspect cases (Signorini et al., 2013, Budihal and Perwez, 2014, Bajani et al., 2003). The Immunoglobulin M (IgM) ELISA has improved sensitivity and specificity over the IgG ELISA for leptospirosis at all stages of disease (Signorini et al., 2013). Unlike other infectious diseases, the development of IgG antibodies in leptospirosis patients is highly variable which makes it unsuitable for use in diagnostics (Adler et al., 1980, WHO, 2003). IgM antibodies specific for different serovars have been shown to persist for up to 6 years (Cumberland et al., 2001). Figure 1.5 shows ELISA IgM and IgG persistence for leptospirosis patients (Cumberland et al., 2001).



A1 – Acute phase 1, A2 – Acute phase 2, C – Convalescent, Units - years

Figure 1.5 IgM and IgG persistence in leptospirosis patients (Cumberland et al., 2001)

1.5.3. Q Fever

Q fever is an underreported zoonotic disease caused by the bacteria *Coxiella burnetii* (Maurin and Raoult, 1999). The organism has a worldwide distribution with cattle, goats, and sheep being the primary reservoirs for human infection (Kaplan and Bertagna, 1955, McQuiston et al., 2002, Raoult and Marrie, 1995, Marmion, 1959). As with other zoonoses, there is very little information about Q fever in Kenya since it was first described in Nakuru in 1955 (Craddock and Gear, 1955). There was a recent report of Q fever being the cause of disease in misdiagnosed febrile patients in western Kenya (Knobel et al., 2013).

Infected animals shed the organism in urine, faeces, milk, or placental fluids (Maurin and Raoult, 1999). The organism is persistent in the environment and transmission is primarily through exposure to animal birth products, aerosols from materials contaminated by infected animals, and ingestion of milk (Raoult and Marrie, 1995, Marmion, 1959, van Woerden et al., 2004, Bernard et al., 2012, De Lange et al., 2014). *C. burnetii* has been isolated from ticks but this vector does not play a significant role in transmission to people (Maurin and Raoult, 1999, Eklund et al.,

1947). *C. burnetii* is a very infectious organism with one bacterium able to cause infection in people (Brooke et al., 2013).

Outbreaks of Q fever in slaughterhouse workers have been documented in countries worldwide and slaughterhouse workers have been shown to have a higher seroprevalence than people in the community (Van Peenen et al., 1978, Marmion, 1959, Carrieri et al., 2002).

The majority of human infections are asymptomatic, whilst some people develop mild disease such as a nonspecific flu-like illness (Maurin and Raoult, 1999). The presenting signs are normally fever, sweating, chills, cough, joint pain, muscle pain, headaches, atypical pneumonia, and hepatitis (Brown et al., 1968, Wilson et al., 2010, McQuiston et al., 2002, Maurin and Raoult, 1999). If untreated, chronic Q fever can result in endocarditis (Maurin and Raoult, 1999). Immunofluorescence antibody is the reference technique for diagnosis of Q fever but for seroprevalence studies IgG Phase 2 ELISAs are recommended (Raoult and Marrie, 1995, Blaauw et al., 2012, Waag et al., 1995).

1.5.4 Rift Valley fever

Rift Valley fever (RVF) is a zoonotic arbovirus affecting livestock and people in Africa and the Arabian peninsula (King et al., 2010). Epidemics of RVF are associated with greater than average rainfall and are characterised by abortion in livestock and febrile illness in people (Davies et al., 1985, King et al., 2010). RVF virus has not previously been reported in western Kenya although epidemics have occurred in neighbouring regions (Figure 1.6) (Murithi et al., 2011). It has been suggested that the virus can be maintained in animal populations between epidemics

and potentially spread to new areas through animal movement (LaBeaud et al., 2007).

The virus is transmitted between animals by mosquitoes, but the most common route of infection for people is exposure to infected animals or their products, particularly abortion material during an epidemic when affected animals are shedding large amounts of virus (Anyangu et al., 2010, Mohamed et al., 2010). Slaughterhouse workers are at risk of exposure to infected materials such as blood through cutting animals' throats and handling animal parts (Mohamed et al., 2010, Nguku et al., 2010, Abu-Elyazeed et al., 1996).

The majority of people infected by RVF suffer mild or subclinical disease (WHO, 2010). Fever, nausea, and vomiting are the most commonly reported clinical signs in people (Kahlon et al., 2010, Madani et al., 2003). Other signs include large joint arthralgia, diarrhoea, jaundice, right upper quadrant pain, delirium, neurological manifestations, and haemorrhagic disorders (Madani et al., 2003, Kahlon et al., 2010).

Diagnosis of RVF may be hindered because of the similar presentation to other endemic febrile illnesses such as malaria or other arboviral diseases such as dengue (Kahlon et al., 2010, Shieh et al., 2010). Diagnosis of RVF is made by virus isolation or polymerase chain reaction (PCR) in the early stage of clinical disease (Sall et al., 2002). Virus neutralisation assays are the gold standard of antibody detection, but the requirement for live virus makes their use limited (OIE, 2014). ELISAs for IgM and IgG can be used for diagnosis and surveillance of RVF (Paweska et al., 2005).

1.5.5. Taeniasis

There are two human tape worms found in east Africa, *Taenia saginata* and *Taenia solium.* They have different intermediate hosts, cattle and pig respectively. Both are transmitted to people by eating undercooked meat from infected animals. Kenya is considered a highly endemic area for *T. saginata* (Hall et al., 1981, Urquhart, 1961). In contrast, *T. solium* is an emerging zoonotic threat in the region (Mafojane et al., 2003).

Slaughterhouse workers are not specifically at risk for taeniasis due to its mode of transmission. However, the author hypothesised that workers may be more likely to eat infected meat and hence have a higher prevalence of disease. This conjecture has been demonstrated in a study in Poland that showed meat handlers were 14 times more likely to have a tapeworm infection than those in other professions (Gemmell, 1983).

Clinical signs of tapeworm infection are generally asymptomatic but adult worms can cause abdominal pain, nausea, debility, weight loss, flatulence, diarrhoea, or constipation due to the presence of the worm in the gut of the host (Craig and Ito, 2007). Taeniasis by *T. solium* has less overt signs, as the tapeworm is smaller and less motile (Gemmell, 1983).

Diagnosis of tapeworm infection in the definitive human hosts can be made by faecal examination (Gemmell, 1983). An alternative method for detecting *Taenia* infection is the coproantigen ELISA which has better sensitivity than microscopy but does not differentiate between *Taenia* sp (Allan and Craig, 2006).

1.5.6 Cysticercosis

Cysticercosis is the larval form of the human tapeworm *Taenia solium*. Human cysticercosis occurs when people ingest tapeworm eggs in food or drink contaminated by a human tapeworm carrier and *T. solium* larvae develop in the tissues (Pan American Health Organization, 2003). The intermediate host is the domestic pig and increases in pig production across East Africa in recent years have led to an increase in porcine and human cysticercosis (Phiri et al., 2003, Mafojane et al., 2003).

Similarly to taeniasis, slaughterhouse workers are not necessarily a high risk group for cysticercosis. The author proposes that due to their increased access to infected meat those slaughterhouse workers may have a higher prevalence of disease.

Infection with the larval form in humans can result in neurocysticercosis which is considered the leading cause of acquired epilepsy in developing world (Carpio et al., 1998). This occurs because the infection is cleared from most tissues by the immune system but the ocular and neural tissue is protected from the immune system and hence cysts can develop unhindered (Garcia and Del Brutto, 2005). Disease develops with the death of the larvae, resulting in an immune reaction causing non-specific pathology of the nervous system (Pan American Health Organization, 2003).

Diagnosis of neurocysticercosis is made by computerised tomography (CT) and magnetic resonance imaging (MRI) to detect lesions (Garcia and Del Brutto, 2005). For serological diagnosis, the enzyme-linked immunoelectrotransfer blot (EITB) and ELISA are most commonly used for diagnosis of cysticercosis although the EITB is too labour intensive and costly for field use (Willingham and Engels, 2006).

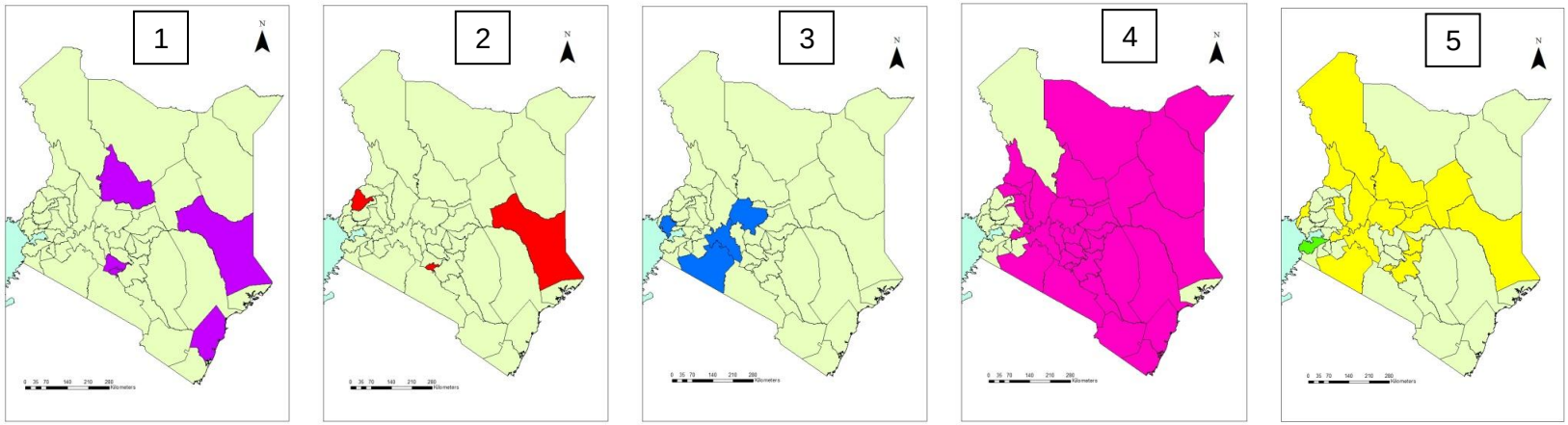
1.5.7 Zoonoses in Kenya

Table 1.2 summarises the zoonoses that are of interest to this study that have been reported in Kenya. Maps of the location of the disease reports are shown in Figure 1.6.

Disease	Species	County	Year	Reference
Brucellosis	Humans	Garissa	2005	Ari et al., 2011
	Cattle	Kiambu, Samburu, Kilifi	1991	Kadohira et al., 1997
Leptospirosis	Humans	Garissa	2005	Ari et al., 2011
	Humans	Bungoma	2004	WHO, 2004
	Rodents	Nairobi	2008	Halliday et al., 2013
Q fever	Cattle Sheep Goats Camels	Laikipia	2011	Depuy et al., 2014
	Humans	Nakuru	1952	Craddock and Gear, 1955
	Humans, Cattle Goats Sheep	Siaya	2007–2010	Knobel et al., 2013
	Humans	Mara	2000	Potasman et al., 2000
RVF	Humans, Cattle	Baringo, Embu, Garissa, Isiolo, Kajiado, Keiyo-Marakwet, Kericho, Kiambu, Kilifi, Kirinyaga, Kitui, Kwale, Laikipia, Machakos, Makueni, Mandera, Marsabit, Meru, Mombasa, Murang'a, Nairobi, Nakuru, Narok, Nyandarua, Nyeri, Samburu, Taita Taveta, Tana River, Tharaka, Trans Nzoia, Uasin Gishu, Wajir, West Pokot	2007	Murithi et al., 2011
Taenia	People	West Pokot	+	Hall et al., 1981
	People	Baringo	+	Kipyegen et al., 2012
	People	Busia	2000–2009	Kagira et al., 2011
	People Cattle	Turkana	2006	Asaava et al., 2009
	People Cattle	Nairobi, Samburu	+	Wanzala et al., 2003
	Cattle	Uasin, Gishu, Kericho, Nakuru, Narok, Laikipia, Isiolo, Meru, Embu, Machakos, Garissa	+	Onyango-Abuje et al., 1996
Cysticercosis	Pigs	Homa Bay	2010	Eshitera et al., 2012
	Pigs, People	Busia, Kakamega, Nairobi	+	Phiri et al., 2003

+ Year of study not recorded in publication

Table 1.2 Published reports of zoonoses in Kenya

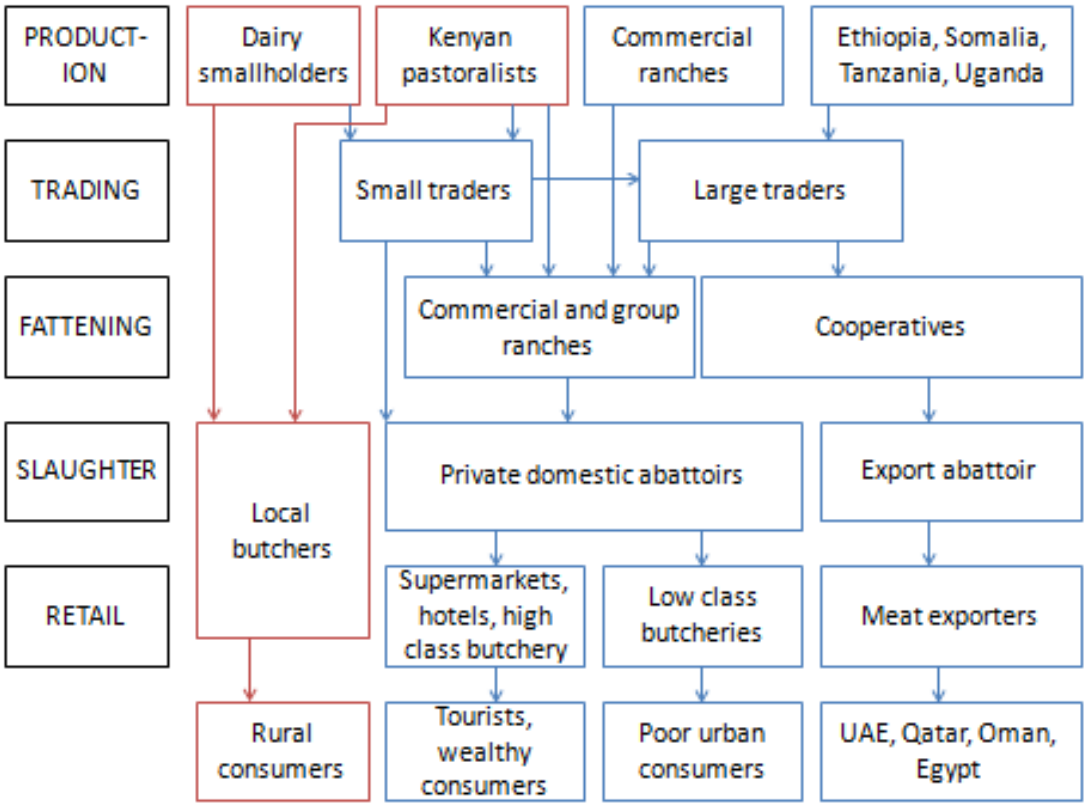


1. Brucellosis
2. Leptospirosis
3. Q fever
4. RVF
5. Taeniasis/ Cysticercosis (green)

Figure 1.6 Counties where zoonotic disease has been reported in Kenya

1.6 The Kenyan meat industry

It is difficult to estimate the number of slaughter facilities in Kenya, as up to one third of Kenya's meat production occurs informally (Farmer, 2012). A report commissioned in 2006 to examine the Kenyan livestock sector estimated the number of slaughterhouses to be 2000 (Muthee, 2006). The majority of publications concerning Kenya's meat industry are focused on the large domestic slaughterhouses in Nairobi (Figure 1.7). This bias is a result of the market for meat in Kenya being predominantly urban and the middle class being the largest consumer (Farmer, 2012).



The rural value chain that is predominant in western Kenya is highlighted in red.

Figure 1.7 Flow chart of the meat value chain in Kenya (Adapted from (Farmer, 2012)

The meat industry is regulated by the Department of Veterinary Services under the Ministry of Livestock and Fisheries (Government of Kenya, 2012). The original Meat Control Act of 1972, last revised in 1977, governed slaughter until 2012. A new revised Meat Control Act was introduced in 2012 to standardise the meat industry across the country (Government of Kenya, 2012).

The revised act provides information to reduce the risk of foodborne disease and protect the consumer. The revised guidelines cover components of the slaughter process such as building structure and layout, equipment, personal hygiene, carcass handling, waste management, and meat inspection. Slaughterhouses are categorised into three categories depending on the size of the slaughterhouse and whether the meat is for local consumption or export out of the community (Table 1.3).

Slaughterhouses are further subdivided into ruminant or pig slaughterhouses, out of respect for the Muslim community. Changes are now being implemented across the country to varying degrees. Introduction of the new regulations is slow in rural areas because of previously inadequate regulation. Abrupt enforcement may result in an increase in the informal market as local meat handlers are unwilling to meet the costs of the improved facilities, as has been reported in other countries (FAO, 2010, Mann, 1984).

The majority of slaughterhouses in rural areas are classified under the new Meat Control Act as Category C (Table 1.3) or more commonly referred to as slaughterslabs. As in other developing countries these facilities are privately owned and rented to butchers who employ their own team of slaughter workers (FAO, 2010, Clottey, 1985). There is a lack of refrigerated transport so meat is sold “hot” or

unrefrigerated in local markets, or is eaten at point of sale as roasted, boiled or fried meat (Figure 1.8) (Farmer, 2012). It is estimated from a study in Nairobi that consumption of meat at the point of sale accounts for 60% of the market (Farmer, 2012). This percentile is supported by data collected during the PAZ study suggesting 65% of people eat meat prepared outside the home (Thomas, 2013). There is a smaller informal market for meat that continues outside the regulatory system that includes "backyard" slaughter (Farmer, 2012). Informal slaughter facilities are not regulated and may contribute to illegal livestock trading and the slaughter of diseased animals (Clottey, 1985).

There are no published reports of the standards in slaughterhouses in western Kenya but a study investigating risk for meat contamination reported that smaller slaughterhouses have poor hygiene practices, unskilled labour, lack of infrastructure, and lack of water (Kariuki, 2013). Figure 1.9 demonstrates the types of slaughterhouses represented in western Kenya.

Figure 1.8 Butchery in western Kenya (A) Metal container for transporting meat (B)

	CATEGORY A	CATEGORY B	CATEGORY C
Animal number	>40 bovines >20 sheep/goats >8 small pigs	6–39 bovines 16–24 sheep/goats 1–7 small pigs	<5 bovines <15 sheep/goats <6 units small pigs
Export meat	Export all Kenya	Export up to 50km	Supply town centre
Location	Any	Not within a city	Not within a city
Land size	2.5 hectares	1.5 hectares	0.5 hectares
Structure		Perimeter fence Floor – water proof concrete Walls and ceiling – Portland cement plaster	
Lighting	Natural and artificial	Natural or artificial	Natural light
Animal holding	Lairage for 2 days	Lairage for 1.5 days	Lairage for the day
Main slaughter hall	Slaughter area Bleeding area Dressing area Area for dehairing pigs	Slaughter area Separate areas to bleed, dress, eviscerate and split Area for dehairing pigs	Slaughter area Areas for bleeding, dressing, hanging, and meat inspection Area for dehairing pigs
Additional rooms	Office Store Meat inspection Offal cleaning Skins/hides Condemned meat	Office Store Meat inspection Offal cleaning Skins/hides	Office Store Area next to the slab for cleaning offal with water and fly screens
Equipment		Knives, Hooks, Spreaders	
Equipment (more specific)	Overhead rail/Hoist Hide puller Freezers -10°C Chiller	Overhead rail/Hoist Hide puller Freezer -10°C Chiller	Chains for hanging Axes
Sanitation		Boots, aprons, wash basins, refuse container, Hot water >82°C Toilet with hand washing facilities 200 Litres potable water per animal	
Pest control	Rodent control and insect electrocuters	Pest control at entrances	Windows with fly screen
Laboratory	Food safety analysis	Food safety analysis	
Disposal of waste	Incinerator or pit Manure shed Blood tank	Incinerator or pit Manure shed Blood tank	Pit Manure shed Blood tank
Manager	Diploma in Food Hygiene or similar	Basic training in food hygiene	Certificate in Food Hygiene
Meat inspector	Diploma in Meat Grading or similar	Diploma in Meat Grading or similar	Trained as Meat Inspector
Training	3 food safety training sessions a year	2 food training sessions a year	2 food training sessions a year

Table 1.3: Classification of slaughterhouses in Kenya (Government of Kenya, 2012)

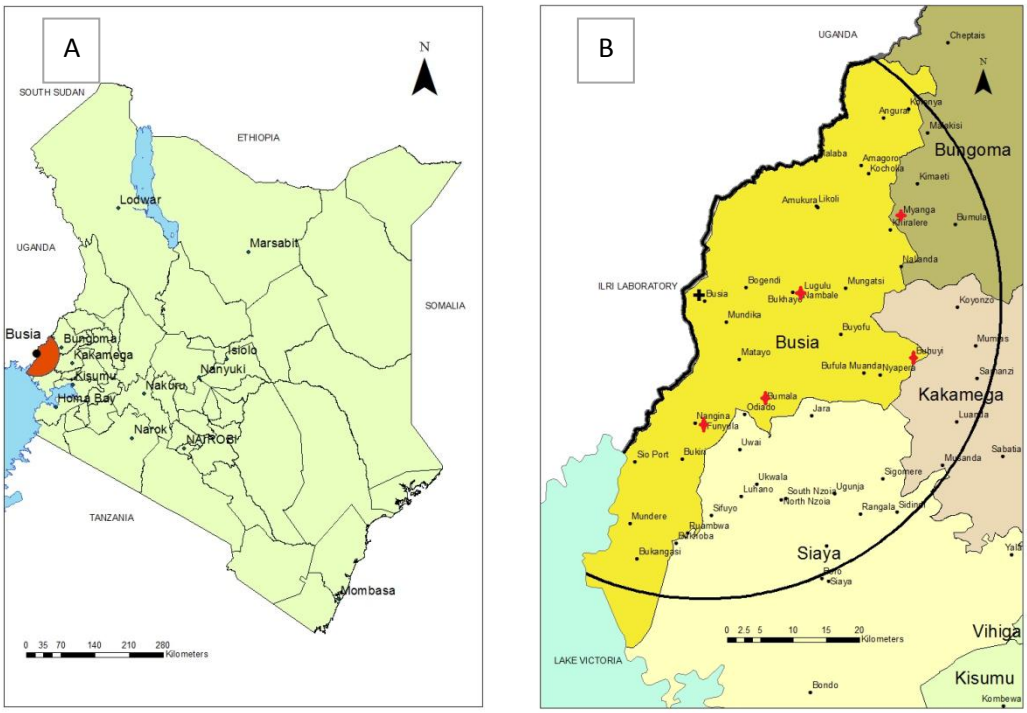
Figure 1.9 Photographs of the types of slaughterhouses in western Kenya

Chapter 2

Study design

2.1 Study site

The study was conducted in western Kenya in the Lake Victoria Basin region of Kenya on the border with Uganda. The study area was a 45 kilometre radius from the project laboratory in Busia (Figure 2.1). This area covered portions of four counties – Busia, Bungoma, Siaya, and Kakamega.



+ Red crosses indicate the main cattle markets

Figure 2.1 Map of study area in western Kenya

The region is predominantly rural with a population of 1.4 million (estimated from the Kenyan Human Population Census of 2009). It is densely populated with a population density of approximately 500 people per square kilometre (Figure 2.2) (estimated from the Kenyan Human Population Census of 2009). The predominant ethnic groups are Luhya, Luo, and Teso. It is estimated more than 40% of homesteads are below the poverty line (Thornton, 2002).

Houses in the study area are made of mud or bricks with thatched roofs or iron sheets (Adazu et al., 2005). The mean homestead size is 5 persons (estimated from the Kenyan Human Population Census of 2009). Mixed subsistence farming is the predominant source of livelihood for 75.6% of homesteads (Adazu et al., 2005).

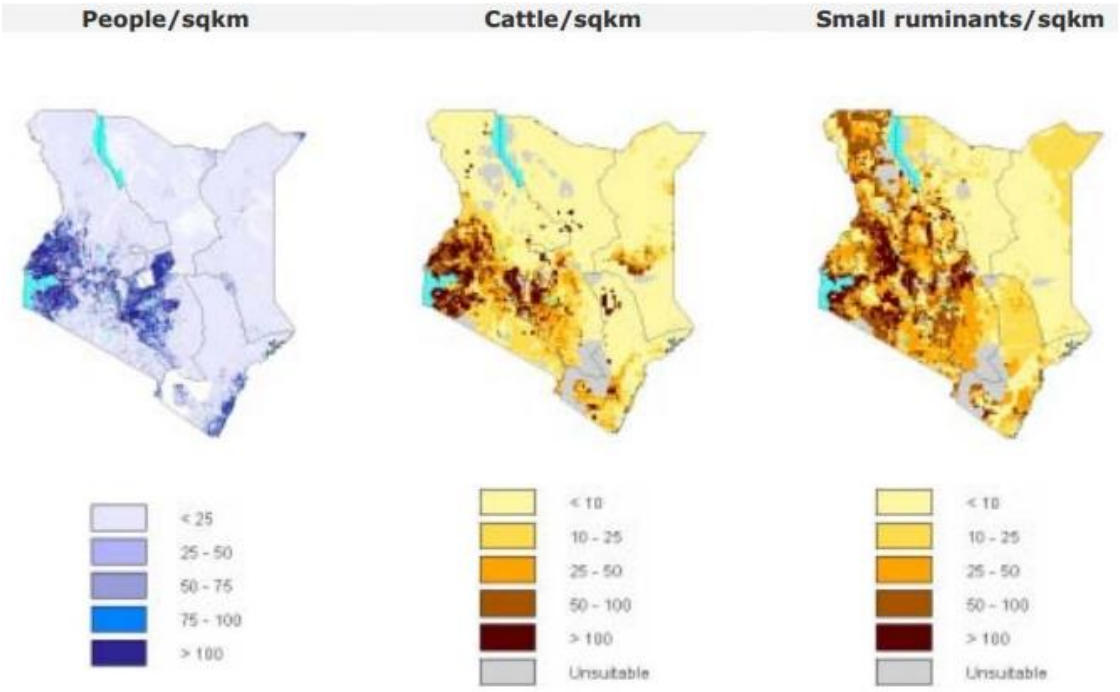


Figure 2.2 Maps showing human, cattle and small ruminant density in Kenya (FAO, 2005)

2.1.1 Justification for research

This study site was chosen for the PAZ project, a cross sectional study investigating zoonotic disease exposure in livestock and non-livestock keeping households, because it is a rural area with a high human and animal population density (Figure 2.2) (Doble and Fevre, 2010). An ongoing Health and Demographic Surveillance System (HDSS) in the region has conducted a number of studies investigating endemic human disease including HIV, tuberculosis, diarrhoeal disease and malaria (Odhiambo et al., 2012). The region is endemic for malaria (Noor et al., 2009) and

has a high prevalence of HIV 6.6–13.9% (KNBS, 2010). It was hypothesised that the high prevalence of malaria and HIV in the region might result in an increased risk of coinfection with zoonotic diseases (Doble and Fevre, 2010). Zoonotic diseases have not previously been investigated in the region, and might have an important role in human and animal health in the region. As previously stated, the slaughterhouse worker project developed from a request by slaughterhouse workers to understand the risks of zoonotic disease exposure in this high risk population.

Six zoonotic diseases were selected for inclusion in the study. Brucellosis, leptospirosis, Q fever and RVF were chosen as there is a demonstrated risk to slaughterhouse workers of exposure to these diseases as discussed in Section 1.5. Cysticercosis and taeniasis were also screened as these zoonoses are endemic in the area, are considered neglected zoonoses, and as previously stated the author hypothesised there might be an increased risk to slaughterhouse workers in the region through the increased consumption of infected meat products.

2.2 Ethical approval

Ethical approvals for the slaughterhouse workers and the PAZ studies were granted by the Kenya Medical Research Institute Ethical Review Committee (SCC Protocols 2086 and 1701 respectively). Ethical approval for animal sampling was granted by the Animal Welfare and Ethical Review Body (AWERB) at The Roslin Institute, University of Edinburgh (approval number AWA004). Informed consent was obtained from all participants. Participants were requested to sign or thumb print a consent form (Appendix 1). A copy was retained for the project records and a second given to the participant with contact details for the project staff if there were any

concerns. All participants were assigned a unique identifier and all samples bar-coded for anonymity.

2.3 The sampling and laboratory teams

The field work required a team of six enumerators including the author. These included 3 clinical officers, a community health worker, and an animal health technician. The Busia laboratory team consisted of 5 technicians – haematologist, parasitologist, and two microbiologists assisting the author. The ILRI Nairobi laboratory team consisted of two technicians assisting the author with the serological tests.

2.4 Study population and recruitment

The study population was every slaughterhouse worker in the study area. A census of slaughterhouses was performed between May 2011 and January 2012. This information was collected by visiting butchers in every village market in the study area and enquiring where slaughtering was performed. The slaughterhouse was visited and recruited into the study. Initially all of the 180 slaughterhouses in the study area were recruited to participate in the study. Sampling was conducted between February and October 2012.

2.5 Sampling

2.5.1 Sampling procedure

All slaughterhouses in the study area were visited 3–6 days before sampling for sensitisation and to explain the project objectives. All slaughterhouses were assigned a unique identification number and mapped using the Global Positioning System (GPS) (Dwolatzky et al., 2006).

On the day of sampling, informed consent was obtained from all participants individually (Appendix 1). Inclusion criteria specified all workers, aged over 18 and present at the slaughterhouse on the day of sampling. Due to the time required to process the samples on the day of collection the number of workers recruited from each slaughterhouse was limited to 12 workers. In slaughterhouses with less than 12 workers all willing participants were recruited. In slaughterhouses with greater than 12 workers a random selection of 12 willing participants from the workers present on the day were sampled. Random selection was conducted by assigning each worker a number. All numbers were written on a piece of paper and placed in a container. A piece of paper was drawn from the container. The worker with the number on the piece of paper was recruited. This was repeated until 12 workers had been selected.

Exclusion criteria included third trimester pregnancy, severe anaemia, under the age of eighteen, inebriation, aggression, and extreme old age. A clinical officer from the project team, responsible for all medical examinations, could exclude participants for any underlying health condition where participation might affect them adversely.

All participants were offered treatment for any diagnosed parasite infections, including malaria and faecal parasites. These conditions were reported confidentially to participants who were then treated for these conditions free of charge by the PAZ project.

2.5.2 Data collection

Four data collection tools were used to collect data regarding slaughterhouses and workers.

- A 114-item individual questionnaire was administered to each participant by one of seven trained interviewers (Appendix 2). Interviews were conducted in Kiswahili, Luo, Luhya and English depending on the language in which the participant was most comfortable. Data were collected on personal history (age, gender, marital status, education, etc.), dietary habits, knowledge of zoonoses, risk behaviours, exposure to livestock, and personal hygiene practices at the slaughterhouse. The questionnaire was developed to include risk factors that had previously been reported in the literature as described in Section 1.4.6. Images of zoonotic diseases (bovine tuberculosis, brucellosis, echinococcosis, and cysticercosis in animals and anthrax in people) were used to determine zoonotic disease recognition in workers (Appendix 7).

- An assessment of health status for all participants was made using standard indicators including height, weight, mid-upper-arm circumference (MUAC), self-reported disease episodes, measurement of anaemia, and a physical examination. These health indicators were recorded as part of the worker questionnaire.

- A second 72-item questionnaire was administered to the foremen of the slaughterhouses regarding slaughterhouse structure, equipment, and practices (Appendix 3).

- The interviewer recorded observations regarding practices where slaughtering was observed at the time of interview.

Questionnaires were pretested in 3 slaughterhouses bordering the study area through January 2012. Questionnaire data were recorded in a Palm operating system (Palm OS) personal digital assistant (PDA) using Pendragon Forms 5.1 (Pendragon Software Corporation, Libertyville, IL, USA). Microsoft® Access databases were used to manage data.

2.5.3 Mapping

Slaughterhouses were georeferenced using a handheld GPS device (Garmin eTrex®). The locations of slaughterhouses were mapped using ArcGIS™ version 9.1 and version 10.2.2 (ESRI, Redlands, CA, USA). Maps were provided by the ILRI geographical information systems unit (http://www.ilri.org/gis/). Mapping allowed analysis of the spatial distribution of the slaughterhouses where workers were seropositive for the specified zoonotic diseases.

2.5.4 Biological sample collection

Samples were collected from every slaughterhouse worker that gave informed consent. 14mls of blood was collected by a clinical officer from each participant (10ml plain BD Vaccutainer® and 4ml Ethylenediaminetetraacetic acid (EDTA) BD Vaccutainer®) using a 21G or 23G BD Vaccutainer® Safetylok™ blood collection set. Thick and thin blood smears were prepared from fresh blood by the clinical officers in the field. These were used to screen for malaria and trypanosomiasis. At recruitment participants were given a 30ml container and requested to collect a faecal sample on the morning of the specified sampling day.

2.6 Sample analysis

2.6.1 Parasitological analysis (Busia laboratory)

Samples were transferred to the project laboratory in a cool box within 3 hours of collection. Serum samples were centrifuged at 3000 rpm for 20 minutes and aliquoted in duplicate in Nalgene® 2ml cryovials and stored at -40°C. The EDTA sample was used to determine packed cell volume (PCV) and total protein concentration and then stored at -40°C. 2–3 grams faecal samples were stored in 3–4mls of 5% formol saline with 0.3% Tween 20 at room temperature (24°C). PCV and total protein were measured to determine anaemia.

The parasitological analyses of the samples were performed at the Busia laboratory before the samples were transferred to ILRI, Nairobi. A microhaematocrit tube of EDTA blood was used to measure PCV and light microscopy was used to examine the buffy coat for trypanosomes. The plasma from the microhaematocrit was used to measure total protein using a refractometer. Thick and thin smears were stained with Giemsa stain and examined for haemoparasites – *Plasmodium* and *Trypanosoma* sp. The techniques for these procedures are described in the *District Laboratory Practice in Tropical Countries* (Cheesbrough, 2006). Faecal samples were examined for evidence of intestinal parasitism using the formol ether concentration method described in *Bench Aids for the Diagnosis of Intestinal Parasites* (Ash, 1994). Serum and EDTA samples were periodically transferred on dry ice to ILRI, Nairobi and stored at -80°C.

Figure 2.3 is a flow chart of the processes performed at the Busia laboratory. The highlighted processes are those relevant to this thesis. During the course of the PhD

the author was awarded a Medical Research Council (MRC) Centenary grant to investigate the epidemiology of enteric bacteria in slaughterhouse workers. Although the samples were collected concurrently with this zoonoses study the material does not contribute to this PhD thesis and will be reported elsewhere.

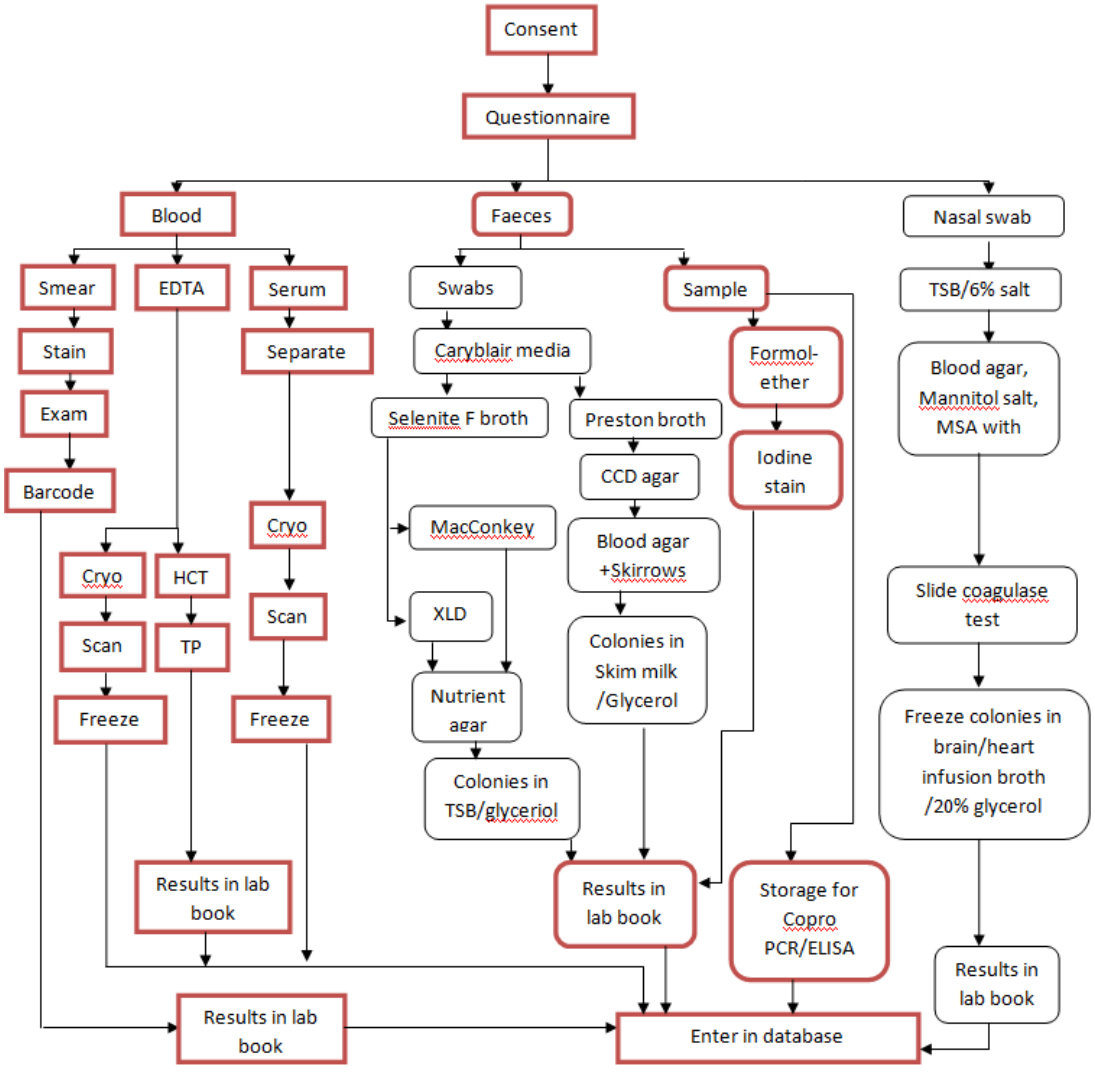


Figure 2.3 Flow chart of the laboratory processes in the Busia laboratory

2.7 Laboratory procedures (ILRI)

Sera were screened for antibodies to *Brucella* sp., *Leptospira* sp, *C. burnetii* and Rift Valley fever. A coproantigen ELISA was performed on collected faecal samples for evidence of *Taenia* sp. An antigen ELISA was used to screen sera for cysticercosis. The tests are described in detail. The sensitivity and specificity reported for the tests used in this study are documented in Table 2.1 at the end of this chapter.

2.7.1 Brucellosis

The RBT was used to screen sera for brucellosis. The RBT is an agglutination test for IgM and IgG antibodies to *B. abortus* and *B. melitensis*. The test reagents were provided by Ignacio Moriyón, University of Navarra, Spain. The technique has been described by Diaz et al. (Diaz et al., 2011). Briefly, the agglutination test was performed on a glossy white ceramic tile. After bringing the serum and antigen to room temperature 25µL of serum was placed on the tile with a clean pipette. The antigen suspension was homogenised by shaking and 25µL placed beside the serum drop. A fresh toothpick was used to mix the serum and antigen. The tile was then rocked gently for 4 minutes using a timer. Any agglutination was considered a positive result.

2.7.2 Leptospirosis

The Panbio Leptospira IgM ELISA (Alere, Sinnamon Park, Australia) was used to screen the sera for antibodies to *Leptospira* (Winslow et al., 1997). The ELISA is a qualitative test for antibodies to a broad range of *Leptospira interrogans* serovars including: hardjo, pomona, copenhageni, australis, madanesis, kremastos, nokolaevo, celledoni, canicola, grippotyphosa, szwajizak, djasiman, and tarassovi4 (Winslow et

al., 1997). An IgM ELISA was chosen because the specificity and sensitivity of IgM ELISAs for leptospirosis is better than IgG. IgM antibodies persist for up to 6 years where the longevity of IgG antibodies is variable, making it inappropriate for diagnosis (Signorini et al., 2013, Budihal and Perwez, 2014, Cumberland et al., 2001, Adler et al., 1980).

The ELISA was conducted as per the manufacturer's instructions. Sera were diluted 1:100 in the kit diluent and 100µL of diluted sample, controls, and calibrator (in triplicate) were pipetted into microwells precoated with *Leptospira* antigen. The plates were covered and incubated at 37°C for 30 minutes and then washed with buffer 6 times. 100µL of conjugate was added to each well and plates were covered and incubated at 37°C for 30 minutes and washed with buffer 6 times. Finally 100µL of substrate was added to each well and plates were incubated at room temperature for 10 minutes. 100µL of stop solution was added to all wells and the plates read at 450nm with a reference filter of 630nm.

The cut–off value is the mean of the triplicate calibrators multiplied by the calibration factor, which is batch specific. The calibrators are calibration agents supplied by the manufacturer.

$$Cut-off\ value = Mean\ of\ 3\ calibrators \times Batch\ specific\ calibration\ factor$$

An index value is calculated by dividing the sample absorbance by the cut-off value.

$$Index\ value = \frac{Sample\ absorbance}{Cut-off\ value}$$

A diagnostic value referred to as "Panbio units" is calculated by multiplying the index value by 10. The results are classified as below:

Index	Panbio units	Results
<0.9	<9	Negative
0.9–1.1	9–11	Equivocal
>1.1	>11	Positive

2.7.3 Q fever

The Serion ELISA Classic *Coxiella burnetii* Phase 2 IgG kit (Virion/Serion, Würzburg, Germany) is a quantitative test that was used to screen the sera for antibodies to Q fever (Peter et al., 1988). Seroprevalence studies for Q fever require testing for IgG Phase 2 antibodies in serum as this is indicative of past infection (Blaauw et al., 2012, Waag et al., 1995). IgG phase 2 antibodies persist longer than phase 1 antibodies and can be present for over 5 years (Dupuis et al., 1985, Waag et al., 1995). Microtest plates were provided with *C. burnetii* antigen. Samples were diluted 1:500 in diluent and 100µL of diluted samples and control sera pipetted into wells. Plates were incubated at 37°C for 60 minutes in a moist chamber then washed 4 times with buffer. 100µL of conjugate was added to wells and incubated at 37°C for 30 minutes in a moist chamber and then plates were washed 4 times. 100µL of substrate was added to wells and plates incubated at 37°C for 30 minutes in a moist chamber. Finally 100µL of stop solution was added to wells and plates read at 405nm and 630nm.

A correction factor, which was calculated by dividing the reference optical density (OD) of the standard serum with the current OD of the standard serum, was used to account for interassay variability.

$$Correction\ factor\ = \frac{OD_{reference\ values\ of\ the\ standard\ serum}}{OD_{current\ value\ of\ standard\ serum}}$$

All measured values of samples were multiplied by the correction factor. The corrected ODs could then be compared to the batch specific cut-off provided by the manufacturer. Corrected OD values below 0.38 were considered negative, OD values over 0.541 were considered positive and those in between these values were borderline.

It is recommended that borderline cases be tested with a repeated serum sample. As this was not possible for the purposes of this study, borderline cases were classified as negative as recommended by the manufacturer.

2.7.4 RVF

The BDSL cELISA is an inhibition ELISA used to detect RVF antibodies (BDSL, Dreghorn, Scotland) (Paweska et al., 2005). Plates were coated with 100µL capture antibody and incubated overnight at 4°C then washed 3 times. 200µL of blocking buffer was added to each well and incubated at 37°C in a moist chamber for 60 minutes. Test and control sera were diluted in duplicate 1:10 with virus and control antigen and then 100µL of test and control sera with virus antigen were added to rows A-D 1-12 and 100µL of test and control sera with control antigen were added to rows E-F 1-12 and incubated at 37°C in a moist chamber for 60 minutes then plates were washed 3 times. 100µL of detection antibody was added to wells and incubated

in a moist chamber at 37°C for an hour and plates were washed 3 times. 100µL of conjugate was added to wells and incubated in moist chamber at 37°C for an hour and plates were washed 6 times. 100µL of substrate was added to wells and incubated in the dark at room temperature for 30 minutes. 100µL of stop solution was added to wells and plates read at 405nm. The net OD for each serum is calculated by subtracting the OD reading of the control antigen from the OD of the virus antigen. The result is converted to a percentage inhibition (PI) using the equation

$$Percentage\ inhibition\ = \left(\frac{net\ OD\ of\ test\ sample}{net\ OD\ of\ negative\ control}\right) \times 100$$

The cut-off is determined by the manufacturer to be 38.6PI.

2.7.5 Taeniasis

A coproantigen ELISA was used to detect tapeworm antigens in human faeces (Allan et al., 1996). This diagnostic test does not differentiate between carriage if *T. solium* or *T. saginata*. The reagents were supplied by the Dorny Laboratory at the Institute of Tropical Medicine, Antwerp, Belgium. Faecal samples preserved in formol ether as described previously were mixed equal volumes with phosphate buffered saline (PBS) (approx 2ml of each). This mixture was left to sit for 1 hour with intermittent shaking. Samples were centrifuged for 30 minutes at 2000 rpm. The supernatant was then aliquoted into a 2ml Eppendorf tube. A Nunc Maxisorp (VWR) plate was coated with 2.5 µg/ml polyclonal IgG in 0.05M carbonate/bicarbonate coating buffer (Sigma, Gillingham, UK). 100µl was pipetted into each well and the plate incubated at 37°C for one hour whilst shaking. Plates were washed once with PBS/0.05% (v/v)

Tween 20. Plates were blocked with 150µl of PBS/0.05% (v/v) Tween/ 2% (v/v) new born calf serum (Invitrogen, Life Technologies, Ltd, Paisley, UK) at 37°C for one hour whilst shaking then emptied. 100 µl of sample, negative and positive controls were added to wells and incubated shaken for one hour at 37°C . Plates were washed five times. Biotinylated polyclonal 2.5µg/ml with blocking buffer (100µl) was added to wells and plates incubated shaken for one hour at 37°C. Plates were washed five times. Streptavidin (Jackson Immunoresearch, West Grove, PA, USA) 1:10,000 in blocking buffer was added to wells (100µl) and incubated for one hour at 37°C whilst shaking. Substrate was prepared from Ortho-Phenylenediamine (DAKO, Ely, UK) and 0.04% (v/v) 30% hydrogen peroxide and added 100µl added to wells and incubated for 15 minutes at 30°C in the dark without shaking. Plates were stopped with 0.5M sulphuric acid solution (50µl) and read at 492 and 655nm.

A cut-off value was determined using 64 negative samples from people sampled as part of the PAZ project, who lived in the study area and never ate pork, with no history of tapeworm and negative for *Taenia spp.* on three microscopic tests). The cut-off of 0.874 was the mean + 3 standard deviations as described by Allan et al. (Allan et al., 1996). The mean OD from each duplicate sample was calculated and the ELISA result determined by applying the above cut-off value to the mean ODs.

2.7.6 Cysticercosis

An antigen ELISA was used to detect *T. solium* cysticercosis in people (Harrison et al., 1989). The HP10 ELISA was supplied by Leslie Harrison at the University of Edinburgh. ELISA plates (Immulon 1, Thermo Life Sciences, Horsham, UK) were coated with 100µl of 0.05M carbonate/biocarbonate coating buffer (Sigma) with

McAb-HP10 (10µg/ml), covered and incubated overnight at 4°C, then washed twice with 0.9%(w/v) NaCl-0.05%(w/v) Tween 20. The plate was blocked using 200µl of PBS/1%(w/v) Bovine serum albumin/0.05%(w/v) Tween 20 added to each well and left for 1 hour at room temperature. Plates were washed 3 times. 100µl serum was added to the wells and plates incubated for one hour at 37°C. Plates were washed 3 times. Biotinylated-McAB (1µg/ml) in PBS/Bovine serum albumin/Tween was added to the plate at 100µl/well and incubated for 1 hour at 37°C. The plates were washed 3 times. Streptavidin peroxidise conjugate (Sigma) 1:10,000 in PBS/Bovine serum albumin/Tween was added 100µl per well and incubated for 1 hour at 37°C and the plates washed 3 times. 100µl of 3,3′,5,5′-*Tetramethylbenzidine (*TMB) (Sigma) substrate was added and the plate incubated at room temperature for 15–30 minutes. Sulphuric acid solution 0.2M was used to stop the plates and plates were read at 450nm.

A correction factor was used to correct for interplate variations. The correction factor was applied to plates that used the same positive and negative control sera over the screening period. The correction factor was calculated from the first plate run in a series that used the same negative and positive control. The mean OD from the positive and negative controls from the first plate were used to create the correction factor using the equation below and applied to all subsequent plates.

$$Correction\ factor\ = \frac{P_0 - N_0}{P_t - N_t}$$

Where:

P_0 = Mean of the positive control sera from plate 1
N_0 = Mean of the negative control sera from plate 1
P_t = Mean of the positive control sera from plate on test
N_t = Mean of the negative control sera from plate on test

2.7.7 Human Immunodeficiency Virus (HIV)

Samples were tested for HIV using the SD Bioline HIV-1/2 Fast 3.0 test strips (Standard Diagnostics Inc, Korea) (Vijayakumar et al., 2005).

Disease	Name of test	Country	Number tested	Sensitivity	Specificity	Reference
Brucella	**Rose Bengal Test**	**Spain**	**208 (positive) 1559 (negative)**	**87.4**	**100**	**Diaz et al., 2011**
Leptospirosis	**Panbio Leptospira IgM ELISA**	**USA**	**252**	**96.5 (87.9–99.6)**	**98.5 (95.6–99.7)**	**Panbio, 2008**
		Thailand	218	90.8	55.1	Desakorn et al., 2012
		Laos	372	60.9	65.6	Blacksell et al., 2006
		Australia	41	100	93	Winslow et al., 1997
		Hawaii	379	35 (25–46)	98 (96–99)	Effler et al., 2002
		UK	200	90	94	Zochowski et al., 2001
Q fever	**Serion ELISA classic *Coxiella burnetii* Phase 2 IgG**	**Germany**	**511**	**93.4**	**98.5**	**Serion, 2012**
		Canada	152	80	>99	Waag et al., 1995
		Switzerland	213	94.8	100	Peter et al., 1988
RVF	**BDSL Rift Valley Fever Inhibition ELISA**	**Kenya, Tanzania,** South Africa**,** Uganda	**1367**	**99.47**	**99.66**	**Paweska et al., 2005**
Taenia	**Coproantigen ELISA**	**Zambia**	**817**	**84.5(61.0–98.0)**	**92.0(90.0–93.8)**	**Praet et al., 2013**
		Guatemala	1582	98	99.2	Allan et al., 1996
Cysticercosis	**HP10 Antigen ELISA**	**Mexico**	**116**	**84.8**	**94**	**Fleury et al., 2007**

Table 2.1 Sensitivity and specificity reported for the diagnostic tests in this study. The sensitivity and specificity used for this thesis are highlighted.

CHAPTER 3

Cross–sectional survey of slaughterhouses and slaughterhouse workers in western Kenya: comparison between ruminant and pig slaughterhouses

3.1 Introduction

Slaughterhouses are places where animals are slaughtered for food (Stevenson, 2013). Internationally there are recognised guidelines for minimum standards in slaughterhouses regarding structure, sanitation, and hygiene practices (FAO, 2010, Codex Alimentarius Commission, 2005). These regulations are to ensure a safe product for consumers and reduce the risk of disease transmission from animals to people (Mann, 1984).

In Kenya a new Meat Control Act was enacted in 2012 (Government of Kenya, 2012). The Act aims to standardise the meat industry and provides guidelines on the required infrastructure and facilities for slaughterhouses in Kenya. Changes to slaughterhouses across the country began in 2012.

This study was conducted before the implementation of the new Meat Control Act and aimed to qualify and quantify the standards in slaughterhouses in rural western Kenya and to make recommendations for areas of necessary and immediate action.

The hypotheses of the study are that:

1. slaughterhouses in western Kenya have inadequate infrastructure and poor sanitation
2. slaughterhouse workers in western Kenya have poor hygiene practices
3. slaughterhouse workers are aware of zoonotic diseases

3.2 Methods

3.2.1 Study area

The study was conducted in rural western Kenya in the Lake Victoria Basin region bordering Uganda (Figure 2.1). The study area and population is described in detail in Section 2.1. The study was conducted between May 2011 and October 2012. The recruitment and sampling of slaughterhouse workers is described in detail in Sections 2.4 and 2.5.

3.2.2 Data collection

This project investigated the current practices in slaughterhouses in western Kenya using four tools: 1) a questionnaire related to the facilities and practices within the slaughterhouses as a unit (Appendix 3); 2) observations regarding the slaughterhouse facilities; 3) an individual questionnaire regarding knowledge, attitudes, hygiene practices, and health of the worker (Appendix 2); and 4) a clinical health assessment of the worker.

3.2.3 Data management

Variables were recoded in R software version 3.0.2 (R Core Team, 2013). Variables that were recoded for the purposes of analysis:

- Age was coded into 10 year age groups 18-27, 28-37, 38-47, 48+
- Age was also coded as a binary variable less or equal to the median age of 39 years.

- Variables relating to frequency of an event such as wearing protective clothing were recoded as binary variables to Always/Sometimes versus Rarely/Never.

3.2.4 Data analysis

Descriptive statistics were performed in R software. The *Survey* package (Lumley, 2012) in R was used to adjust for clustering. Weights for the slaughterhouse level data were calculated by dividing the number of each type of slaughterhouse by the number sampled. Weights for the slaughterhouse worker data were calculated by dividing the number of slaughterhouse workers expected in the slaughterhouse by the number sampled. Weights and a finite population correction factor (fpc) were used to calculate a design effect using the *svydesign* function in *Survey* using the following equation. The design effect was used to calculate adjusted proportions for survey responses.

$$designobject <- svydesign(id = \sim slaughterhouse\_id, weights = \sim weights, data = shwdata, fpc = \sim fpc)$$

Variables were analysed for independence using the *svychisq* command in *Survey* which calculated a Pearson's Chi squared statistic adjusted by the design effect. A level of 5% statistical significance (Type 1 error) was used. Graphs were made in Microsoft® Excel 2007 using the design adjusted survey results. Maps were made using ArcGIS™ version 9.1 and version 10.2.2 (ESRI, Redlands, CA, USA).

Multiple correspondence analysis (MCA) was used to assess the relationship between slaughterhouses and selected variables. This method of variable reduction

was used because the data consists of categorical variables (Husson, 2010). The outputs were used to make a two dimensional map which was interpreted as a graphical representation of the relationship between variables and slaughterhouses (Hoffman, 1992). The methodology is described in detail below. Simply a variable is plotted as the central point between all slaughterhouses that have that characteristic. Slaughterhouses are plotted close to characteristics they share and far from characteristics they do not. This graphical representation can be interpreted that the slaughterhouses close to each other are similar to each other. This information can be used to create categories of slaughterhouses that are similar with regard to these variables.

Variables that were included in the MCA are those that best approximate the standard requirements described in Section 1.4 and Table 1.3. Variables included were those that represented:

- structure (roof, sides, floor) – coded present/absent
- sanitation (latrine, hand washing place) – coded present/absent
- hygiene (workers wearing coveralls/boots) – coded always/sometimes/never worn
- meat inspection (antemortem inspection) – coded always/sometimes/never performed
- size of slaughterhouse (number of people) – coded ≤ 3, ≤ 10, >10
- number of animals slaughtered coded ≤ 5, ≤ 10, >10
- type of slaughterhouse (type of animals slaughtered) – coded Cattle, sheep and goats/Cattle only/Pig only

The MCA was developed in the *FactomineR* package (Husson, 2014). The methodology behind this is well described by Greenacre et al. (Greenacre and Blasius, 2006).

In order to perform the MCA a matrix is constructed for the slaughterhouses and the selected variables with each row representing slaughterhouses and each column a variable. A profile is created for each slaughterhouse defined by variable responses and a profile for each variable defined by the slaughterhouse responses. The mean of all profiles is the centroid (centre of gravity). The distance between the centroid and a profile point is the chi-squared distance, which is calculated from the observed and expected values of each profile. The average of all the chi-squared distances is defined as inertia. The points are in multiple dimensions, and this is simplified by selecting the principal dimensions. For the purposes of this study the first 2 dimensions were examined graphically, which explain the maximum amount of inertia possible in 2 dimensions. The eigenvalue is the square root of the inertia, and is used to describe the total variability explained by the dimension.

A dendrogram was created from the MCA output in *cluster* (Maechler, 2013) to group slaughterhouses with similar characteristics based on the coordinates calculated by the MCA (de Souza et al., 2014). This was to determine if classification or ranking of the slaughterhouses was possible for use in future analysis.

3.3 Results

There were 180 slaughterhouses in the study area when the study began in May 2011. 24 slaughterhouses were closed by the District Veterinary Officer between May 2011 and January 2012 for non-compliance with regulations. From the remaining 156 slaughterhouses, 142 slaughterhouses (91%) agreed to participate in the study. Fourteen (9%) slaughterhouses refused to participate. This included 4/57 (7%) cattle and 10/68 (15%) pig slaughterhouses. Although no specific reason was given for refusal, the study team surmised that fear of recriminations from the Department of Veterinary Services was the reason for refusal. Appendix 4 indicates the facilities at the slaughterhouses that declined and also the 24 slaughterhouses that closed during 2011.

Figure 3.1 shows the distribution of slaughterhouses in the study area. There was one cattle and one pig slaughterhouse that used the same facility but for the purposes of analysis, they were considered separate slaughterhouses as the workers were different. The slaughterhouses were evenly distributed throughout the study area. The slaughterhouses that refused to participate were clustered in the south of the study area.

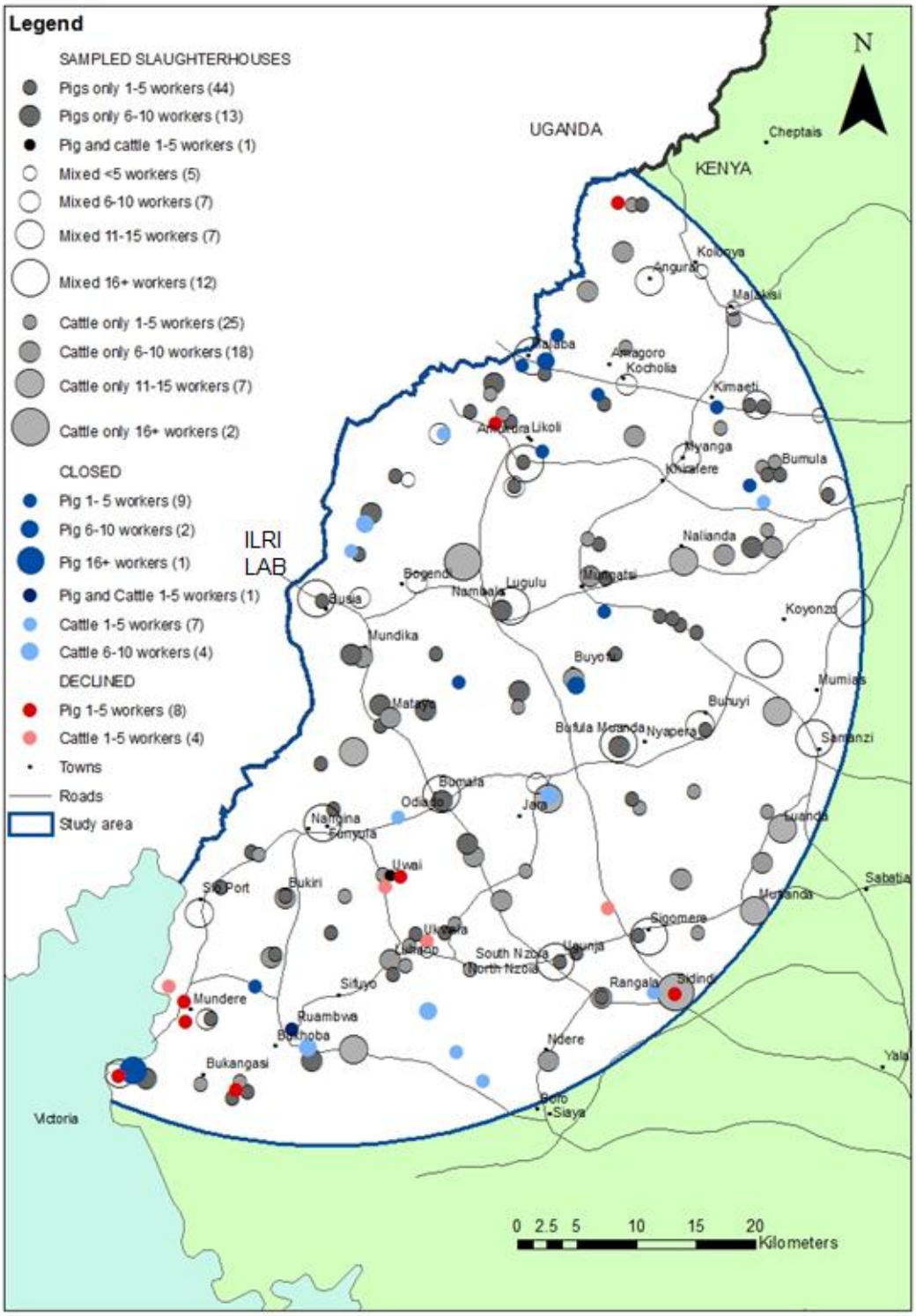


Figure 3.1 Map of slaughterhouses showing location, type and number of workers

Of the 142 slaughterhouses recruited in the study, 31 were mixed ruminant (cattle, goats, sheep), 53 were cattle only and 58 were pig only (Figure 3.2). The total employment at these slaughterhouses was 1005 workers. Workers were interviewed at all 142 slaughterhouses. Questionnaires were administered to 738 workers. Slaughtering was observed at 84 slaughterhouses whilst interviews were being conducted.

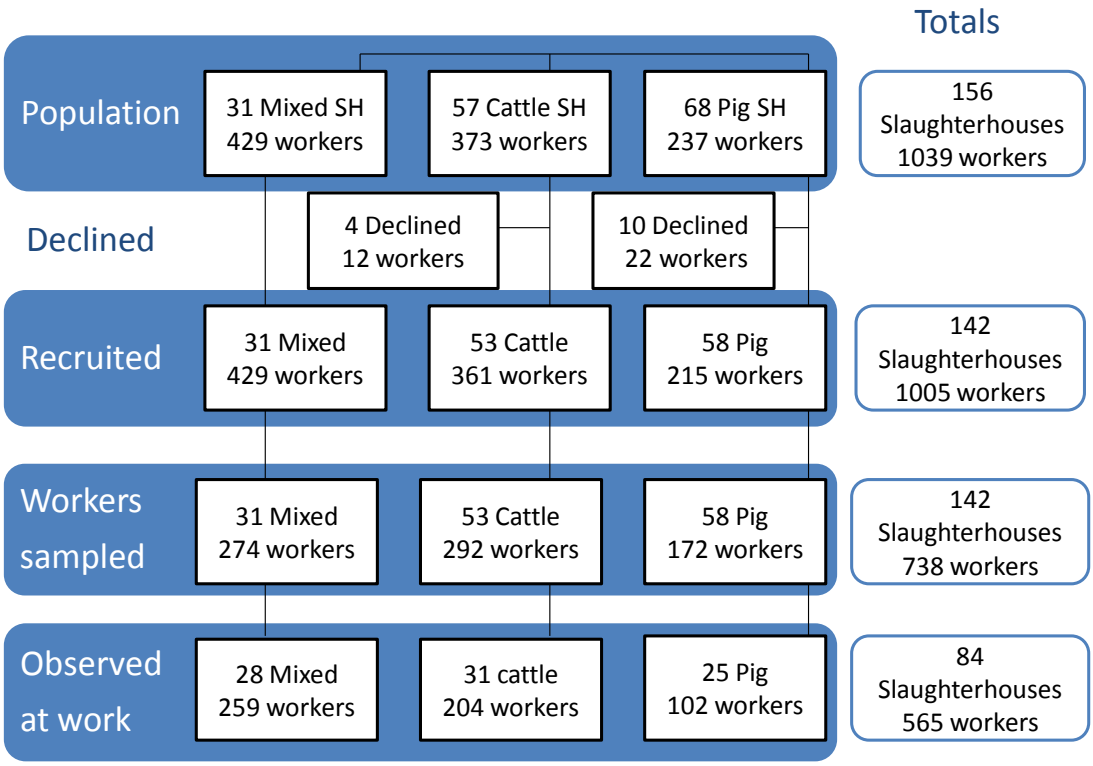


Figure 3.2 Number of slaughterhouses and workers in western Kenya in 2012

Mixed ruminant slaughterhouses had a greater mean number of workers than cattle only slaughterhouses and pig only slaughterhouses, and a greater proportion of licensed workers than pig slaughterhouses (Table 3.1). The approximate number of

animals slaughtered per week across all slaughterhouses was 807 cattle, 404 sheep/goats and 410 pigs.

Variable	Mixed ruminant % n=31^	Cattle only % (95% CI) n=53	Pigs only % (95% CI) n=58	Total % (95% CI) n=142
Average number of workers	14	7 (6–7)	4 (3–4)	7 (6–7)
Percent of licensed workers	61	61 (59–64)	41 (38–45)	52 (51–54)
Average number animals/ week	Cattle 15 Goats 13	Cattle 7 (6–7)	Pigs 7 (6–8)	NA

^ 95% CI not reported for Cattle and sheep/goats as this was a complete census

Table 3.1 Number of slaughterhouse workers and animals slaughtered per week

The results are presented in two parts. The questionnaires completed by the foremen with the observations by the study team form the results regarding the slaughterhouse infrastructure and practices. The questionnaires completed by the workers and the clinical assessment form the results regarding worker practices, knowledge, and health. Tables of the results for all variables of interest are in Appendix 5.

3.3.1 Slaughterhouse infrastructure and practices

a. Slaughterhouse category

Only 2 slaughterhouses were Category B slaughterhouses. The remainder were Category C or informal. It was not possible to determine the informal slaughterhouses, as foremen were unwilling to admit to working outside the regulations.

Despite the vast majority of slaughterhouse classed as Category C, only 26% (95% CI 24–28%) of slaughterhouses restricted meat selling to within the local village,

with the remainder exporting meat outside their immediate area. Figure 3.3 shows the slaughterhouse categories of selected slaughterhouses in western Kenya.

1) Category B Cattle and sheep/goat slaughterhouse 2) Cattle and sheep/goat slaughterhouse - sheep/goat unit 3) Category C Cattle slaughterhouse 4) Category C Pig slaughterhouse

Figure 3.3 Slaughterhouses in western Kenya

b. Slaughterhouse infrastructure and sanitation

Only 65% (95% CI 63–67%) of slaughterhouses were observed to have a roof, cement floor, and solid sides (Figure 3.4). There was a general lack of electricity and piped water, with less than 3% (95% CI 3–4%) of all slaughterhouses having either utility. Overall the foremen reported a lack of toilet facilities 60% (95% CI 57–62%) and hand washing facilities 20% (95% CI 18–22%). These reports were corroborated by the observational results that 60% had toilets (95% CI 52–67%) and 12% (95% CI 7–16%) had hand washing facilities (Figure 3.5). The majority of slaughterhouses, 66% (95% CI 64–68%) sourced water from boreholes. Only 3% (95% CI 3–4%) had piped water with the remainder carrying water in jerry cans to the slaughterhouse (Figure 3.6). A large number of slaughterhouses (78%; 95% CI 76–80%) reported seeing dogs around the facility with a smaller percentage seeing rats (12%; 95% CI 11–14%). Dogs were observed at 83% (95% CI 77–89%) of slaughterhouses. A pit for carcass waste was observed at the majority of slaughterhouses (93%; 95% CI 89–97%).

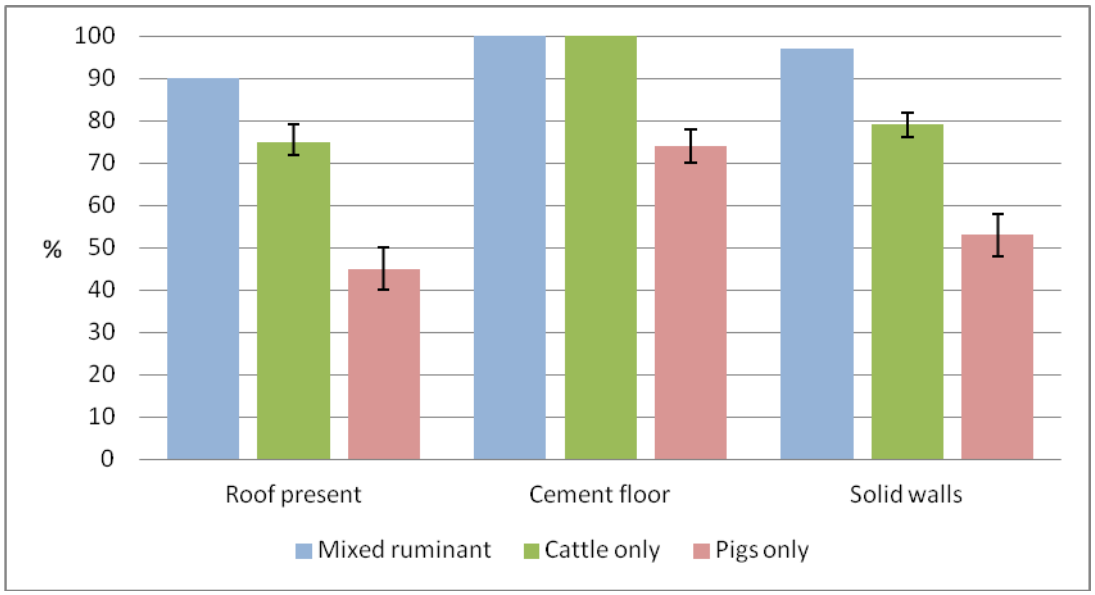


Figure 3.4 Structural features at slaughterhouse – observed

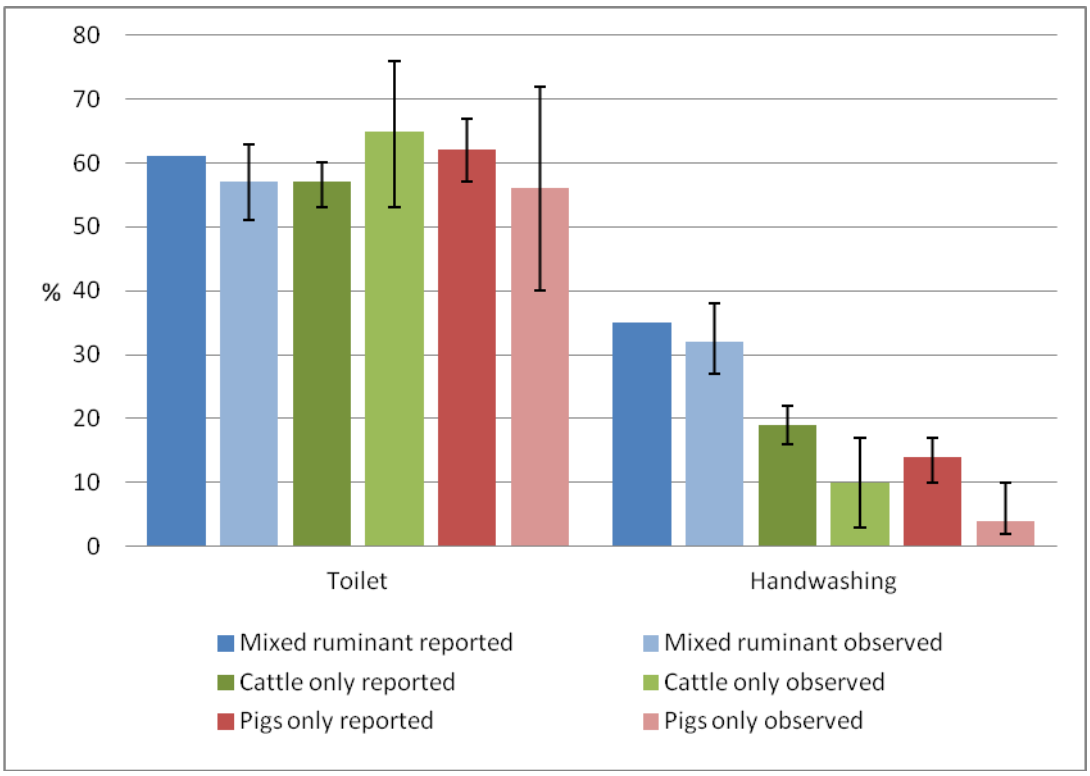


Figure 3.5 Sanitation facilities at slaughterhouses – reported and observed

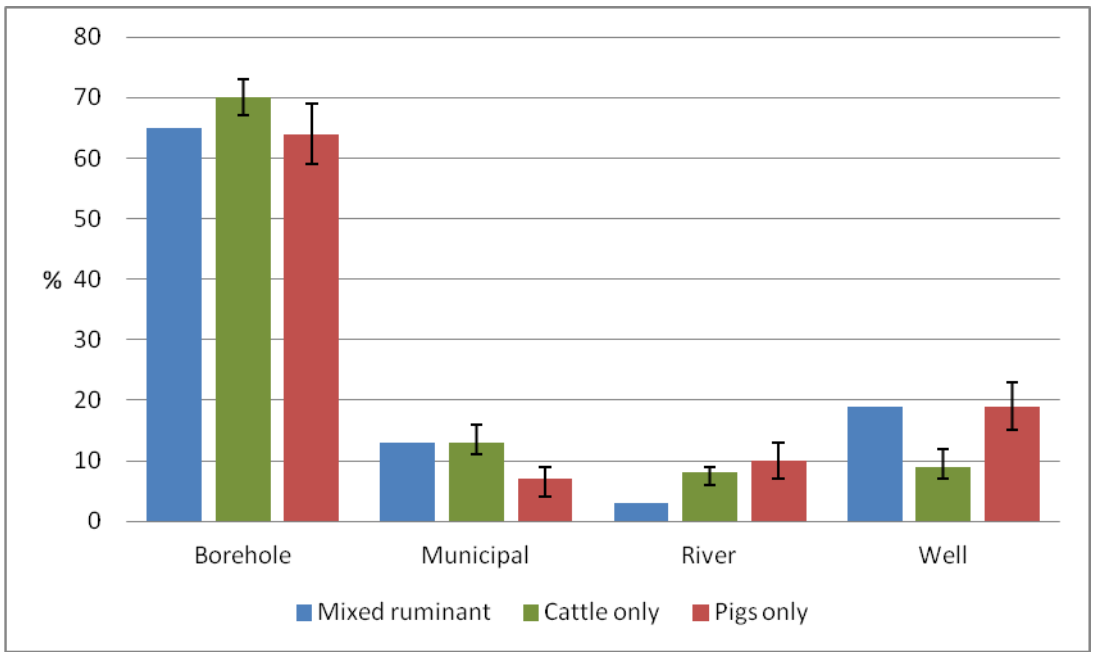


Figure 3.6 Water source at slaughterhouse – reported

Both mixed ruminant slaughterhouses and cattle only slaughterhouses had better infrastructure than pig slaughterhouses. 90% of mixed ruminant slaughterhouses and

75% (95% CI 72–79%) of cattle only slaughterhouses had a roof, cement floor and solid walls compared with 45% (95% CI 40–50%) of pig slaughterhouses (X^2=21.53, df=2 p<0.001).

Slaughtering, bleeding, skinning, and evisceration were performed in the same area in all slaughterhouses. This process is referred to as "batch slaughtering". The viscera were washed outside the slaughterhouse on a concrete slab in all but one slaughterhouse, where there was a specific room inside the slaughterhouse. At only one mixed ruminant slaughterhouse were cattle stunned before slaughter. The remaining 141 slaughterhouses cut the throat instead.

c. Personal hygiene practices (slaughterhouse level)

Less than half of slaughterhouses reported that workers wore personal protective clothing. Workers in 32% (95% CI 29–34%) of slaughterhouses were reported to wear lab coats. 34% (95% CI 31–36%) of slaughterhouses reported that workers wore boots. This report was supported by the observational data that workers in 27% (95% CI 21–34%) of slaughterhouses wore lab coats and workers in 22% (95% CI 17–28%) of slaughterhouses wore boots (Figure 3.7).

Very few slaughterhouses provided protective equipment for workers, with workers providing their own lab coats in 78% (95% CI 73–84%) of slaughterhouses and workers providing their own boots in 84% (95% CI 78–89%) of slaughterhouses. No workers were observed to wear gloves. Workers in mixed ruminant (55%) and cattle slaughterhouses (36%; 95% CI 32–39%) were more likely to wear lab coats than workers in pig slaughterhouses (17%; 95% CI 13–21%) (X^2=13.38, df=2 p=0.001). Workers in mixed ruminant slaughterhouses (52%) and cattle slaughterhouses (45%;

95% 41–48%) were more likely to wear boots than workers in pig slaughterhouses (16%; 95% CI 12–19%) (X^2=16.33, df=2 p<0.001).

Soap was reported to be provided at 64% (95% CI 62–67%) of slaughterhouses but was only observed in 21% (95% CI 15–27%). Soap was observed in 16% (95% CI 7–25%) of cattle only and 12% (95% CI 2–22%) of pig only slaughterhouses. This was significantly less than in mixed ruminant slaughterhouses where soap was observed 50% (95% CI 44–56%) of the time (X^2=4.75, df=2 p=0.001). Eating was observed in 18% (95% CI 12–24%) of slaughterhouses.

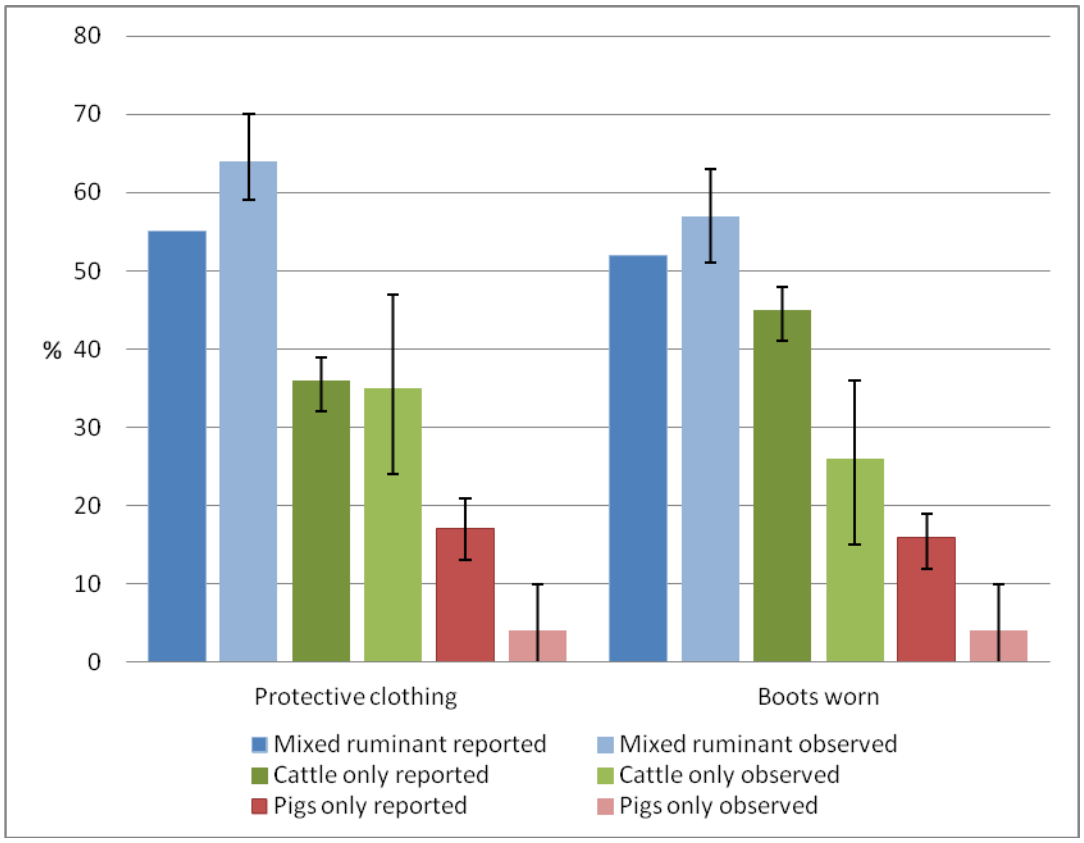


Figure 3.7 Personal hygiene practices at slaughterhouses – reported and observed

d. Meat inspection practices

90% (95% CI 92–95%) of slaughterhouses reported that the meat inspector visited every day. However the meat inspector was seen at only 53% (95% CI 45–61%) of

slaughterhouses. Workers explained that the meat inspector may visit the butchery to inspect the meat if he was too late arriving and did not see the meat at the slaughterhouse. Antemortem inspection was reported at 7% (95% CI 6–8%) and observed at 6% (95% CI 3–10%) of slaughterhouses (Figure 3.8). 9% (95% CI 7–10%) of slaughterhouses reported slaughtering sick animals.

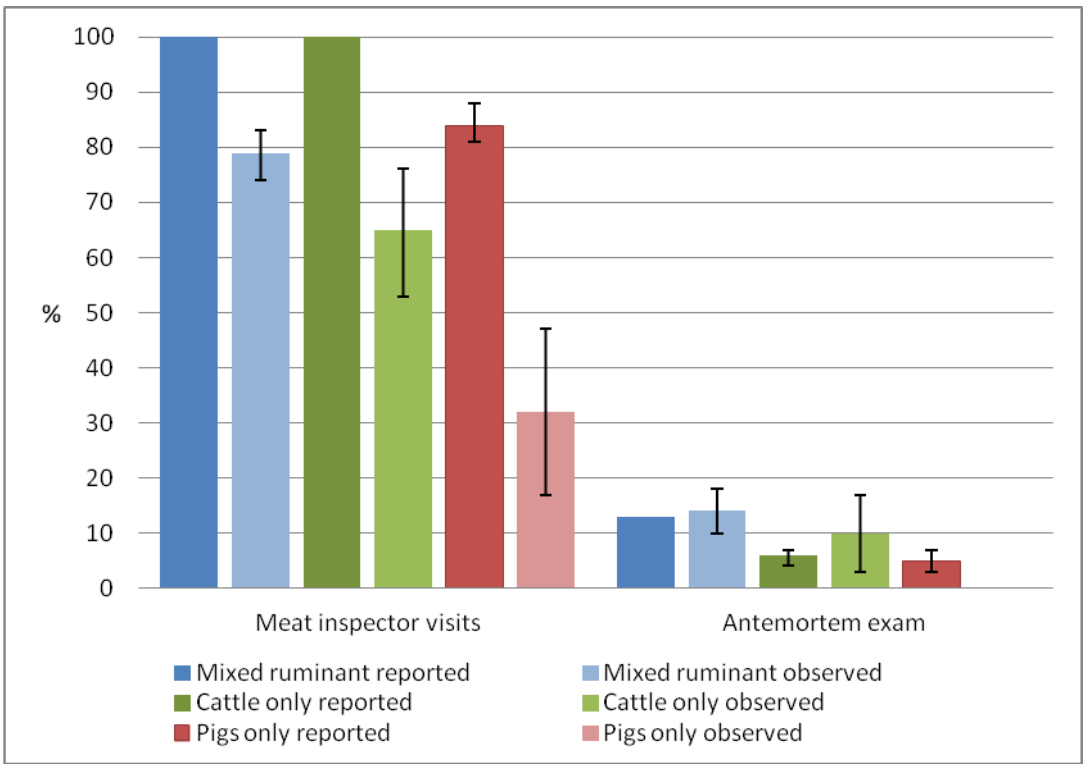


Figure 3.8 Meat inspection practices – reported and observed

3.3.2 Slaughterhouse worker practices, knowledge and health

The 738 slaughterhouse workers ranged in age from 18–82 years with a mean age of 39 (95% CI 39–40). The mean time employed as a slaughterhouse worker was 9.35 years (95% CI 9–10) with a range of 1 month to 59 years. The mean number of days worked per week was 4.9 with a mean work day of 2.5 hours.

The different jobs in the slaughterhouses are demonstrated in Figure 3.9. The slaughterman was responsible for cutting the animals throats in mixed ruminant and cattle slaughterhouses. Flayers were responsible for skinning and sectioning the carcass. There was usually a worker that cleaned the intestines. There was not an official slaughterman in pig slaughterhouses. The same worker that did the throat slitting also sections the carcass. In this study these people were grouped together with flayers. Although the skin of pigs was not removed, the other roles were the same as flayers in the mixed ruminant and cattle only slaughterhouses. There was not an assigned person for the cleaning of the pig intestines as these were not consumed. Cleaners were responsible for cleaning the slaughterhouse.

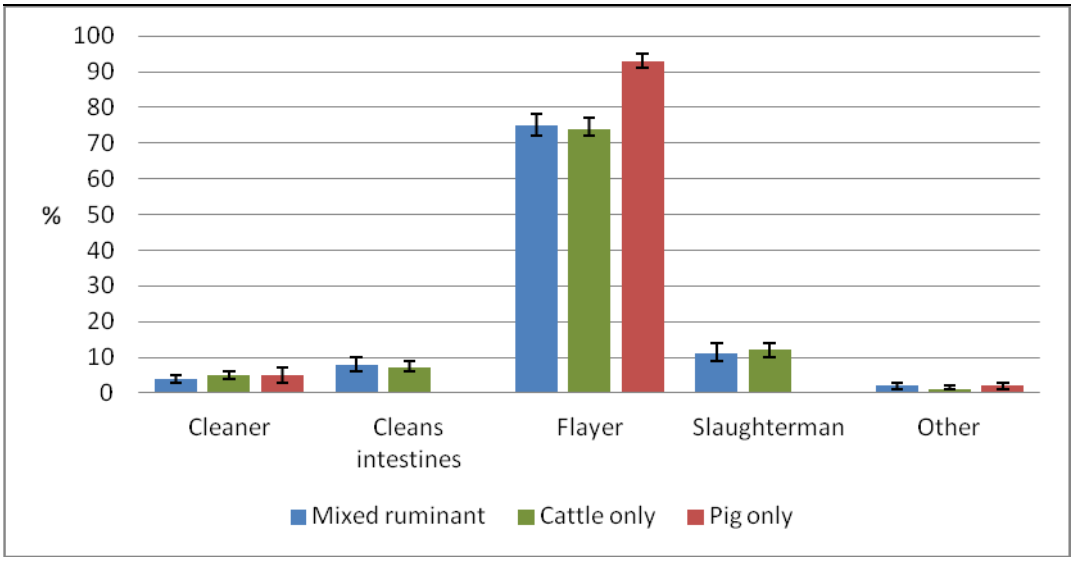


Figure 3.9 The distribution of jobs in the slaughterhouse – reported

97% (95% CI 96–97%) of slaughterhouse workers were men. 74% (95% CI 73–76%) of workers had predominantly primary level education. 82% (95% CI 80–83%) of workers had a second occupation, predominantly as butchers (42%; 95% CI 40–44%) (Figure 3.10). 72% (95% CI 70–74%) of workers had contact with livestock outside of work (Figure 3.11). The majority of workers had contact with poultry (88%; 95% CI 86–89%) and cattle (72%; 95% CI 70–74%).

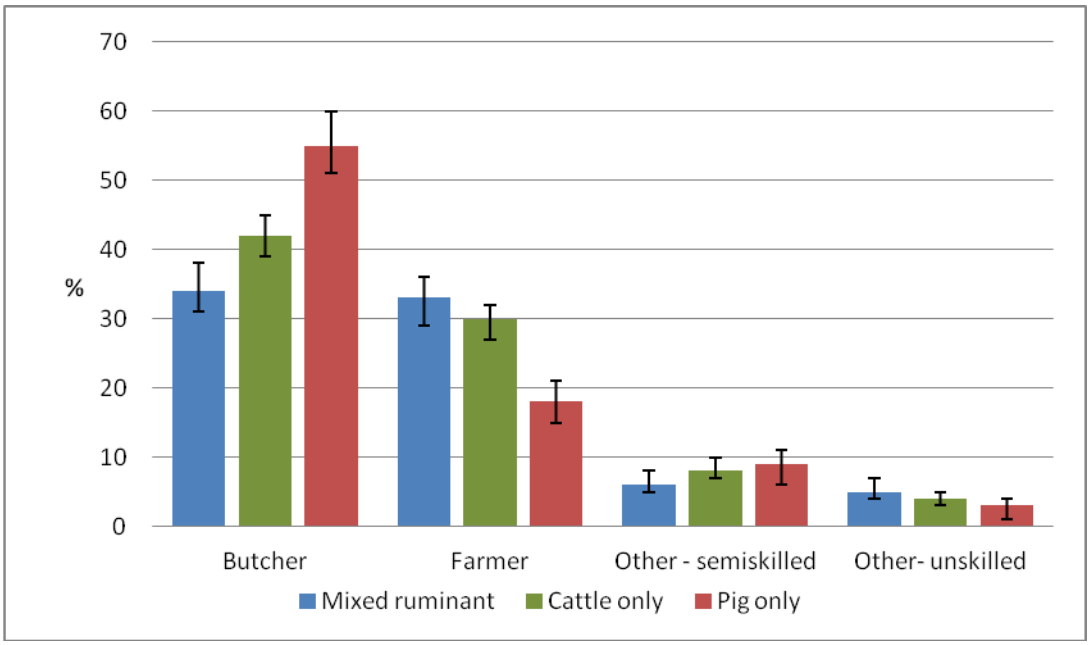


Figure 3.10 Secondary occupations of slaughterhouse workers – reported

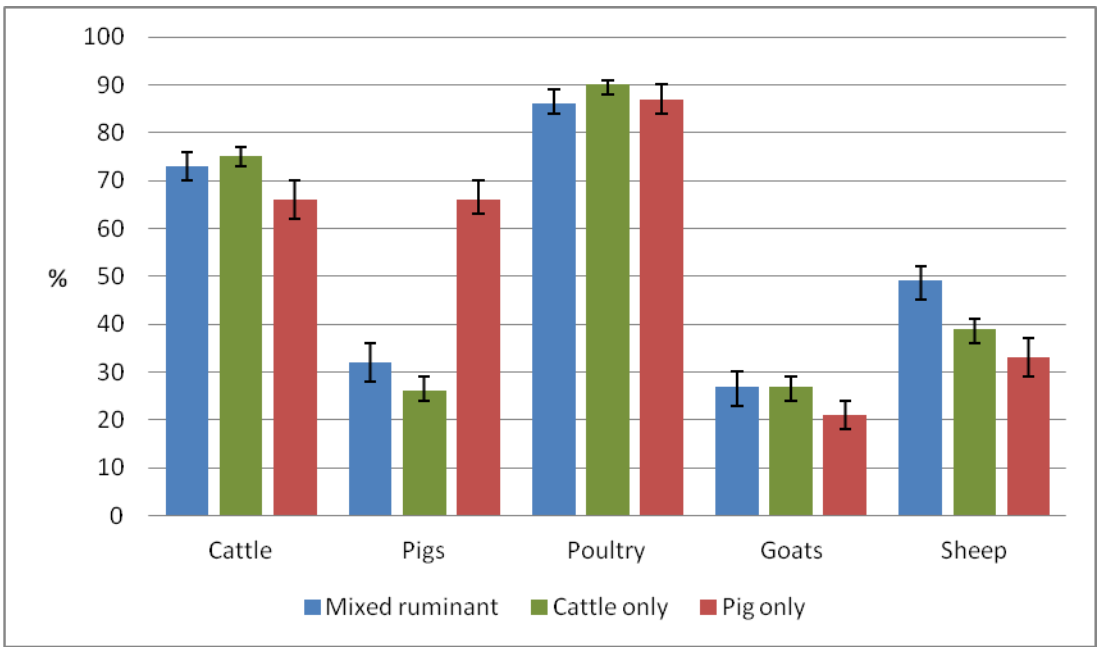


Figure 3.11 Slaughterhouse worker contact with animals outside work – reported

a. Slaughterhouse workers' practices

Table 6 shows the knowledge and practices of the 738 slaughterhouses workers interviewed. 53% (95% CI 51–55%) of workers reported wearing protective clothing. Workers at ruminant slaughterhouses (69%; 95% CI 66–73%) and cattle only slaughterhouses (49%; 95% CI 46–51%) were more likely to wear protective clothing (X^2=79.82, df=2 p<0.001) compared with pig slaughterhouse workers (27%; 95% CI 23–30%). 49% (95% CI 46–51%) of workers reported wearing boots. Workers at ruminant slaughterhouses (68%; 95% CI 64–71%) and cattle slaughterhouses (41%; 95% CI 38–44%) were more likely to wear boots (X^2=95.14, df=2 p<0.001) compared with pig slaughterhouse workers (22%; 95% CI 19–26%) (Figure 3.12).

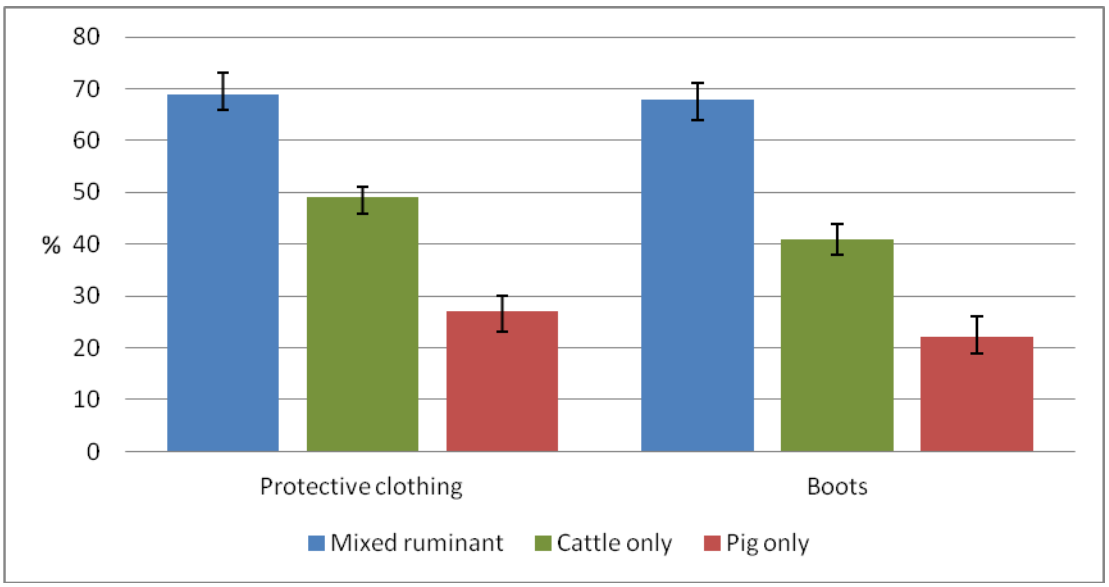


Figure 3.12 Protective clothing and boots worn reported by workers

Almost one quarter of workers smoke daily (23%; 95% CI 21–25%) and 32% (95% CI 30–34%) workers take alcohol daily. The study team observed that 11% (95% CI 10–12%) of workers appeared to be intoxicated at interview (Figure 3.13). 21% (95% CI 21–23%) of workers eat at work (Figure 3.13). At pig slaughterhouses workers were observed to eat pieces of the carcass that were cooked over an open fire. At large mixed ruminant slaughterhouses, there was someone preparing and selling tea to workers. 24% (95% CI 22–26%) of workers reported defecating in the open.

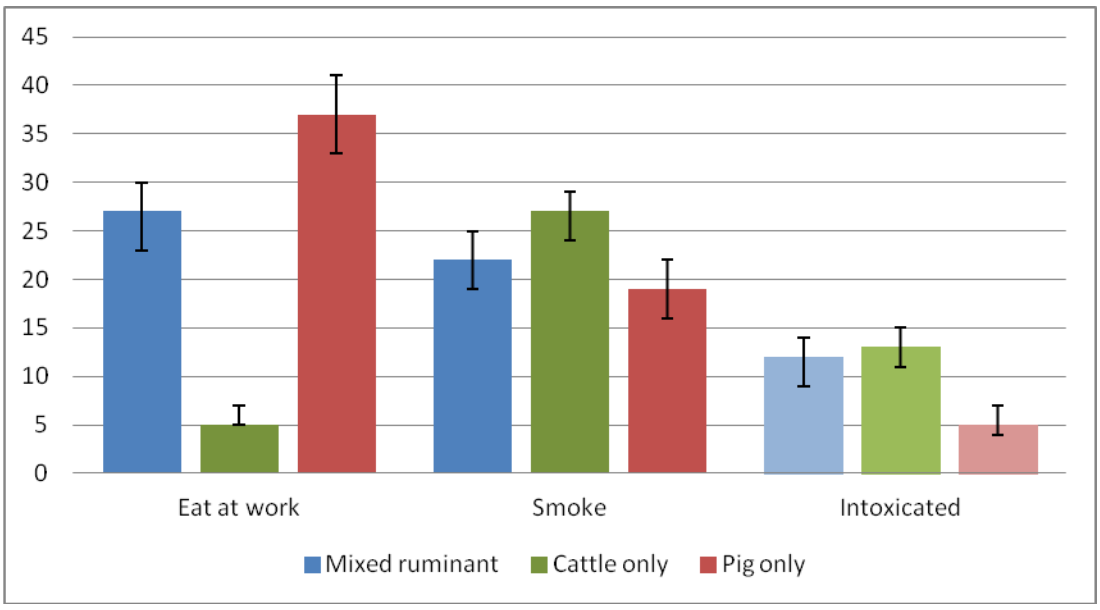


Figure 3.13 Slaughterhouse workers personal hygiene practices – reported and observed

96% (95% CI 95–96%) of slaughterhouse workers reported seeing the meat inspector every day. However, only 44% (95% CI 42–46%) of workers reported the meat inspector performing antemortem inspection of the animals. 18% (95% CI 16–19%) of workers reported slaughtering sick animals (Figure 3.14).

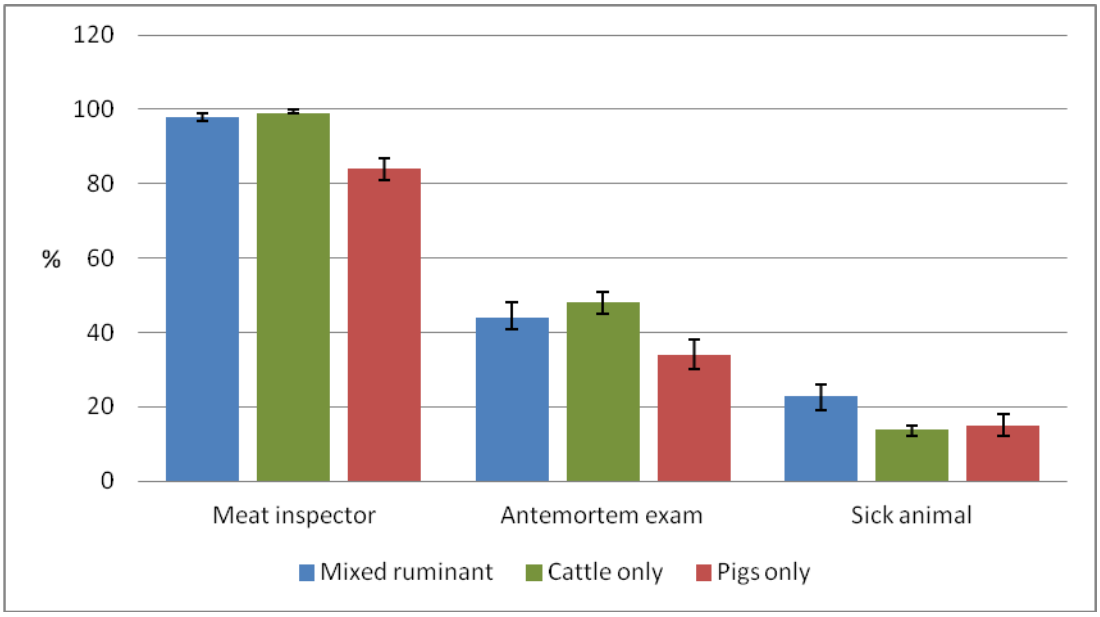


Figure 3.14 Meat inspection practices reported by the slaughterhouse workers

b. Slaughterhouse workers' knowledge

31% (95% CI 29–33%) of the 738 slaughterhouse workers knew that disease can be transmitted from animals. 42% (95% CI 40–44%) knew that meat can be a source of disease. Only 8% (95% CI 7–9%) of workers could name a zoonotic disease (Figure 3.15). Many workers recognised images of tuberculosis, brucellosis, echinococcosis, and cysticercosis lesions in animals but no one correctly named these conditions (Table 3.2).

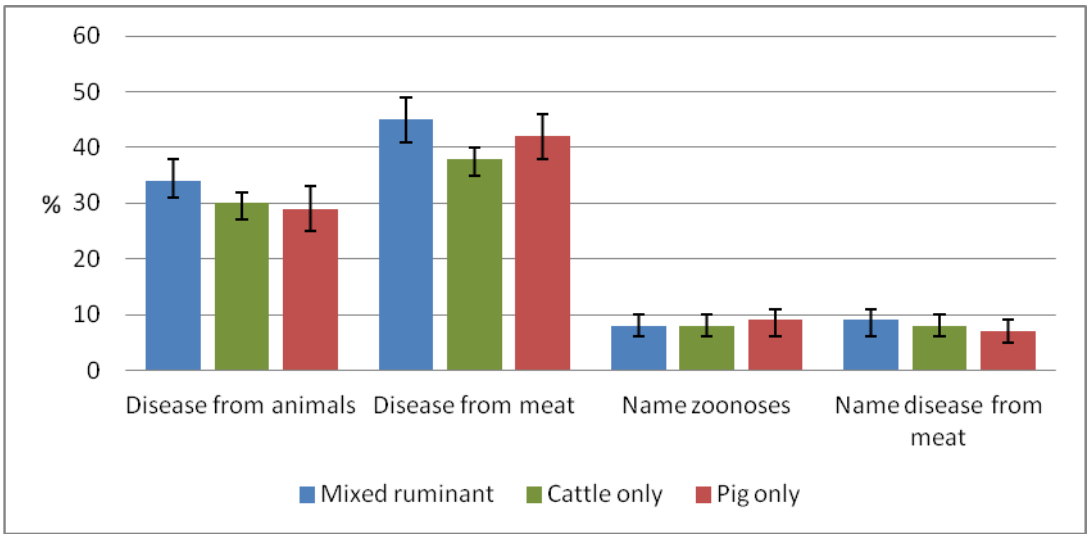


Figure 3.15 Slaughterhouse worker knowledge of zoonotic and foodborne disease

Variable	Mixed % (95% CI) n=274	Cattle % (95% CI) n=292	Pigs only % (95% CI) n=172
Bovine tuberculosis	65 (62–69)	49 (46–51)	NA
Brucellosis in cattle	22 (18–25)	6 (5–8)	NA
Anthrax in people	13 (10–15)	11 (9–12)	NA
Echinococcosis in cattle	33 (30–37)	33 (31–36)	NA
Cysticercosis in pigs	NA	NA	20 (16-23)

Table 3.2 Slaughterhouse workers that recognise images of zoonotic disease in animals

c. Slaughterhouse worker health

18% (95% CI 16–19%) of workers reported being unwell in the past 3 months. Figure 3.16 shows the range of reported symptoms. Fever and headache were the most commonly reported symptoms.

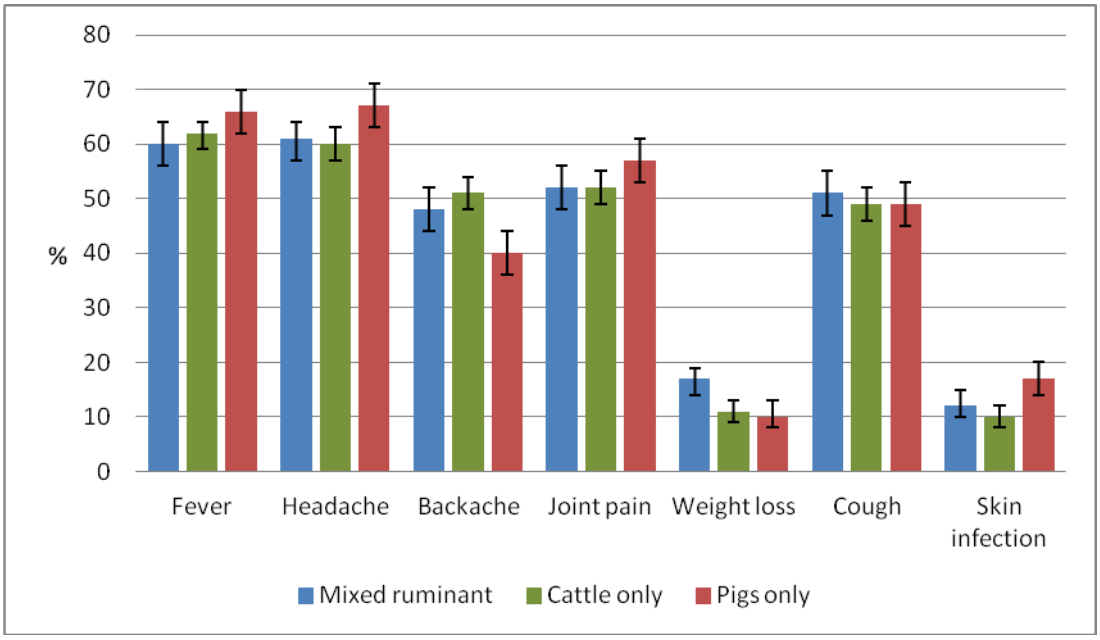
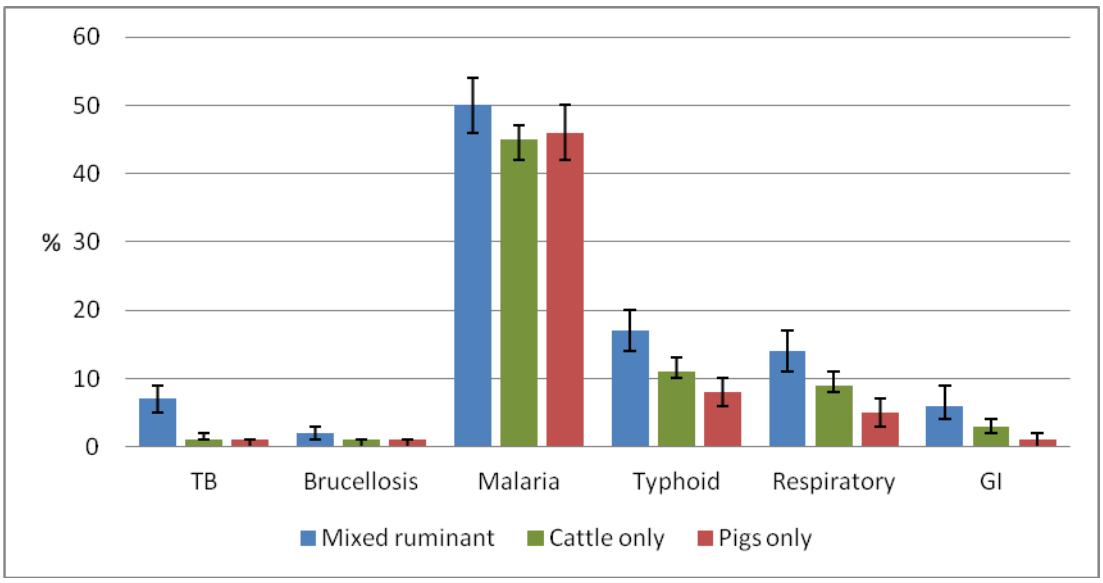


Figure 3.16 Self reported symptoms in the 3 months before interview – reported

Workers at mixed ruminant slaughterhouses were more likely to report typhoid (X^2=8.32, df=2 p<0.001), respiratory illness (X^2=10.11, df=2 p<0.001), or gastrointestinal illness (X^2=6.18, df=2 p<0.001) than cattle only and pig only slaughterhouse workers in the past 12 months (Figure 3.17).

25% (95% CI 23–27%) of workers reported being injured at work at least once a month and 8% (95% CI 7–9%) had a wound at the time of interview (Figure 3.18).



TB/Brucellosis = previously diagnosed with tuberculosis/brucellosis,
Malaria/Typhoid/Respiratory/GI = diagnosed in the past 12 months

Figure 3.17 Health status of the slaughterhouse workers in western Kenya at interview.

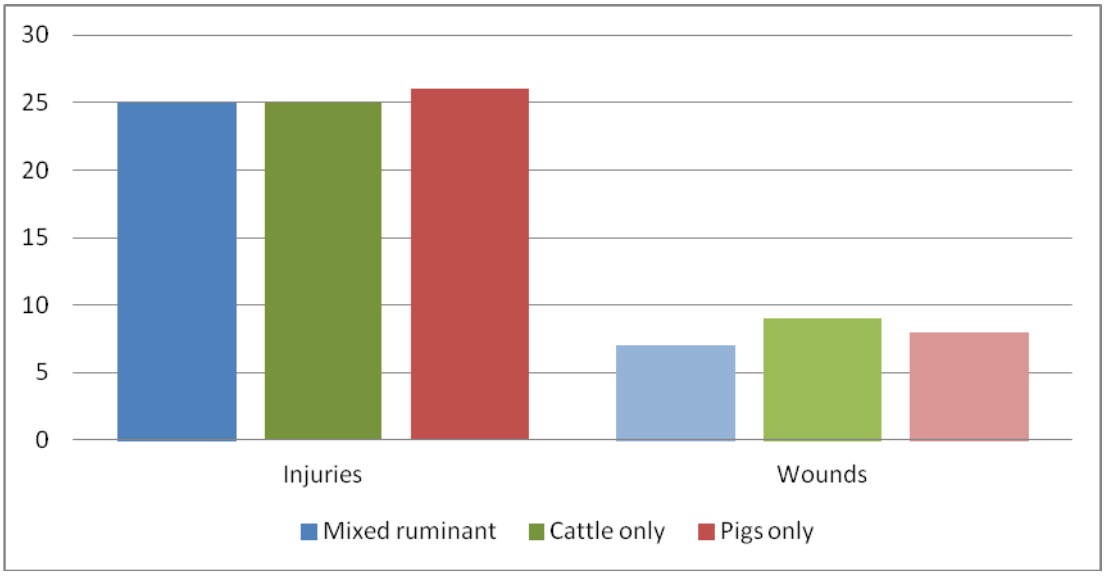


Figure 3.18 Workers that are injured every month and workers with wounds at interview

3.3.3. Multiple correspondence analysis of slaughterhouse data

Figure 3.19 is a graphical representation of the MCA where the proximity of variables can infer similarity. Table 3.3 demonstrates the contribution each variable made to each dimension. A dendrogram was created from the output of the MCA taking the first 5 dimensions which accounted for 59% of the variability in the data. The dendrogram grouped the slaughterhouses into 3 main categories (Figure 3.20).

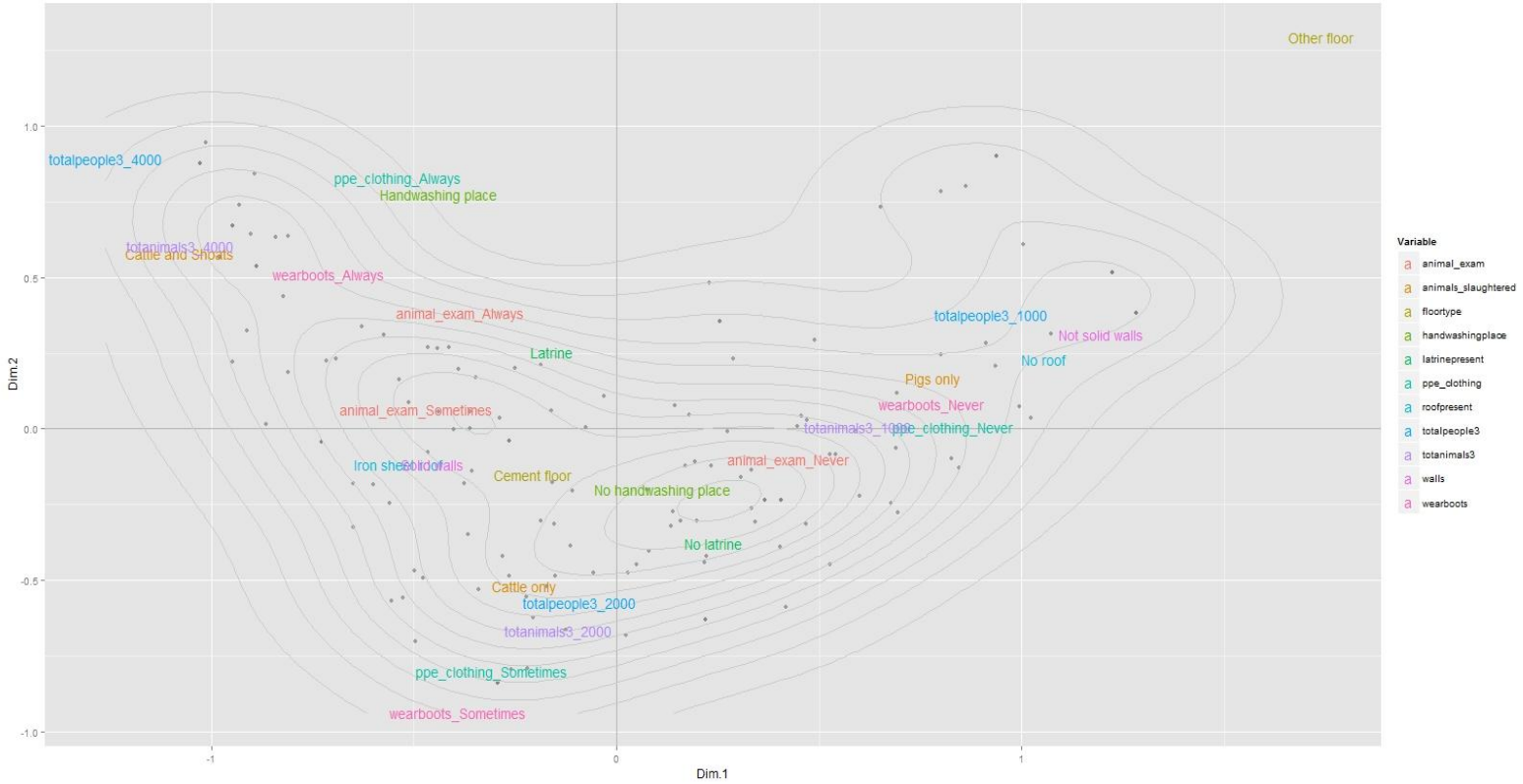
Category 1: predominantly mixed ruminant slaughterhouses, most wear protective clothing and boots, most have a roof and slaughter >10 animals per week

Category 2: approximately 50% pig and cattle slaughterhouses, have a roof and slaughter ≤10 animals a week

Category 3: predominantly pig slaughterhouses, do not wear protective clothing or boots, do not have a roof and slaughter ≤5 animals a week

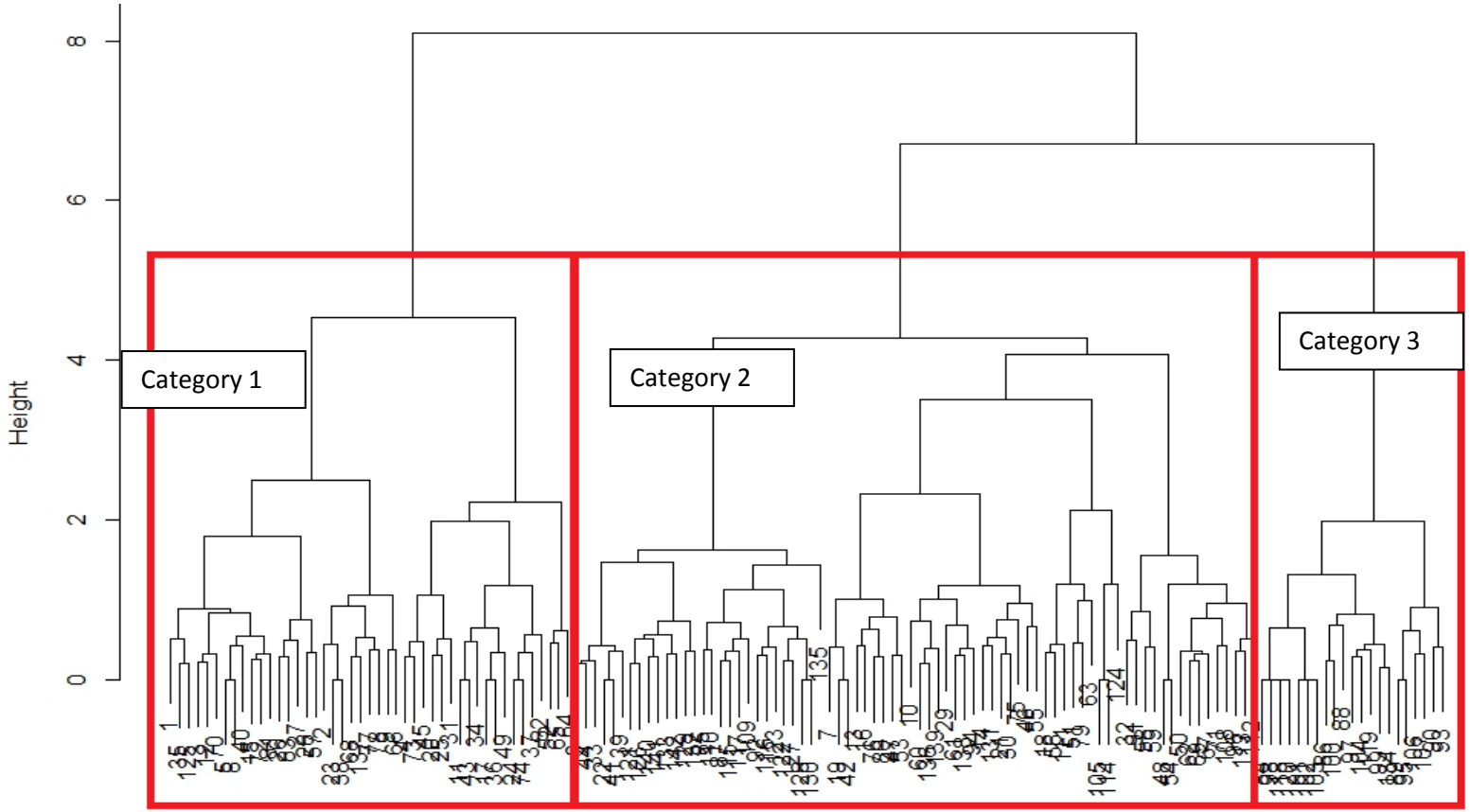
Variable	Dim1	Dim2	Dim3	Dim4	Dim 5
PPE clothing	0.359	0.440	0.218	0.197	0.007
Boots	0.469	0.292	0.225	0.078	0.115
Animals	0.522	0.185	0.298	0.224	0.019
Roof present	0.568	0.027	0.052	0.113	0.000
Hard floor	0.357	0.198	0.007	0.008	0.003
Walls	0.541	0.036	0.074	0.030	0.012
Animal exam	0.204	0.018	0.134	0.049	0.606
Latrine	0.038	0.096	0.091	0.216	0.042
Hand washing	0.050	0.154	0.022	0.038	0.358
Total people	0.570	0.360	0.147	0.064	0.015
Total animals	0.482	0.196	0.123	0.203	0.032
% of variance	24.471	11.774	8.190	7.768	7.116

Table 3.3 Contribution of the variables to 5 dimensions created by the multiple correspondence analysis



animal_exam – antemortem inspection of animals (Always, Sometimes, Never); animals_slaughtered – type of slaughterhouse (Cattle and shoats(sheep/goats), Cattle only, Pigs only); floortype – Cement/Other; handwashingplace – Present/Absent; latrinepresent – Present/Absent; ppe_clothing – ppe worn (Always, Sometimes, Never); roofpresent – Iron sheets/No roof; total people3 – 1000 - ≤3, 2000 - ≤10, 4000 - >10; totanimals3 – 1000 - ≤5, 2000 - ≤10, 4000 - >10 ; Walls – Solid/Not solid; wearboots – workers wear boots (Always, Sometimes, Never)

Figure 3.19 The distribution of variables using the first 2 dimensions of the multiple correspondence analysis



The branches represent slaughterhouses

Figure 3.20 Dendrogram of the first 5 dimensions of the multiple correspondence analysis.

3.4 Discussion

The current conditions in slaughterhouses in many developing countries contribute to the spread of zoonotic diseases (Mann, 1984). The lack of facilities and unhygienic practices lead to contamination of meat and spread of disease to workers and the community (Mann, 1984). This study reports the conditions in slaughterhouses in western Kenya with respect to infrastructure, hygiene, meat inspection, and the knowledge and health of workers.

3.4.1 Slaughterhouse infrastructure and sanitation

The most notable findings were the lack of facilities to ensure adequate meat hygiene. Ideally the floor of the slaughterhouse should be hard concrete and impervious, to reduce dirt in the slaughterhouse and allow drainage and cleaning (Bengtssom, 1988). Similarly, a roof is important to protect the carcass from the weather and to reduce the temperature in the slaughterhouse (Mann, 1984, Bengtssom, 1988). 10% of the 142 slaughterhouses did not have a cement floor and over 30% of slaughterhouses did not have a roof.

There should be a division in the slaughterhouse between the dirty (killing, bleeding) and clean (eviscerating and splitting) operations to prevent carcass contamination (Codex Alimentarius Commission, 2005). All slaughterhouses in the study area performed “batch slaughtering”. This is where an animal is killed, bled, skinned, eviscerated, and split in the same spot (FAO, 2010). In the majority of slaughterhouses, carcass preparation was performed on the ground as seen in the representative photographs (Figure 3.3). These processes can lead to carcass contamination from the skin, the intestines and the ground (FAO, 2010).

International guidelines specify that hot and cold water should be readily accessible for cleaning (FAO, 2004, Codex Alimentarius Commission, 2005). Equipment and workers' hands should be washed with soap and hot water (Codex Alimentarius Commission, 2005, FAO, 2004). This process requires piped water facilities that are only available in a few (3%) slaughterhouses. There was a lack of water, hand washing facilities, and soap in all slaughterhouse types. Hand washing is predominantly used to protect meat from contamination, but is also protective against infection with zoonotic disease in workers (Brown et al., 2011, Campagnolo et al., 2000, Gomes-Neves et al., 2012). The lack of hand washing facilities in the majority of slaughterhouses in western Kenya has public health implications to workers and the wider community. Only 60% of slaughterhouses had access to a toilet, with 24% of workers admitting to open defecation. This behaviour promotes the persistence of zoonotic diseases such as cysticercosis (Mann, 1984). The presence of pests in the slaughterhouse results in disease spread, either through contamination of meat or eating of meat scraps by dogs which can lead to persistence and spread of diseases such as echinococcosis (Brown et al., 2011, Mann, 1984, Bengtssom, 1988).

The MCA was a graphical method of examining the relationship between variables from the slaughterhouse questionnaire (Hoffman, 1992). It can be inferred from this and the Chi squared analysis that mixed ruminant slaughterhouses have improved facilities and practices over the other types of slaughterhouses. The chi squared analysis showed that mixed ruminant slaughterhouses are more likely to have appropriate infrastructure such as a roof and cement floor and to have improved hygiene practices with more slaughterhouses having soap and more workers wearing protective clothing.

The MCA analysis was not useful as a method of variable reduction. The first dimension accounted for only 24% of the variability and this was not considered sufficient for this dimension to be used as a new variable in future analyses. The dendrogram grouped slaughterhouses according to the facilities and practices at each slaughterhouse. This process divided the slaughterhouses into three categories. The categories again support that mixed ruminant slaughterhouses are more equipped than other slaughterhouses with pig only slaughterhouses having the least infrastructure and the poorest practices. The new categories defined by the dendrogram were not used in any future analysis as slaughterhouse type was deemed a sufficient method of categorisation.

3.4.2 Slaughterhouse personal hygiene practices

The purpose of protective clothing within the slaughterhouse is primarily to protect the meat product from contamination but has also been shown to be protective against zoonoses in slaughterhouse workers (Brown et al., 2011, FAO, 2004). Less than 50% of workers wore protective equipment at all times. It is likely that the cost of protective clothing is the limiting factor as the majority of workers must provide their own protective clothing. The income for slaughtering a cow is US$1.10 and the cost of boots and apron US$9.50 and US$5.00 respectively.

3.4.3 Slaughterhouse meat inspection practices

A large amount of the slaughter activities occurred without an inspector present. Workers reported that the meat inspector would inspect the meat later at the butchery. Antemortem inspection, which is essential for preventing the slaughter of sick animals was practiced at very few slaughterhouses. Almost 1 in 5 workers

admitted to slaughtering sick animals. Slaughtering sick animals is a risk factor for infection with zoonoses including anthrax, brucellosis, and leptospirosis (Ray et al., 2009, Peck and Fitzgerald, 2007, Swai and Schoonman, 2009, Brown et al., 2011). The paucity of antemortem inspection may be limited by the number of inspectors. Currently inspectors attend to more than 5 slaughter facilities per day. It is the responsibility of the government to train and provide meat inspectors (FAO, 2010).

3.4.4 Slaughterhouse workers' knowledge

A lack of knowledge regarding the process of meat contamination is the biggest hindrance to improving conditions in the meat industry (Mann, 1984). This study showed that few people were able to recognise or name a zoonotic disease. Training personnel in meat hygiene is essential to improving conditions in slaughterhouses and to reduce bacterial contamination of meat and disease exposure in workers (FAO, 2010, Wamalwa et al., 2012).

3.4.5 Slaughterhouse workers' health

Slaughterhouse workers have been identified in occupational health studies for elevated risk of injury, particularly to the upper extremities (mostly due to lacerations) and back injuries (Pedersen et al., 2010, Burridge et al., 1997). Both backache and wounds were reported by workers. This trend may be the result of poor work practices and training or a lack of appropriate equipment (Burridge et al., 1997, Cai et al., 2005). The population of slaughterhouse workers polled by this study is young and predominantly male and they have worked for a short duration of time in the meat industry. Young men are likely to take higher risks (Hannerz and Tuchsen, 2001). This suggests a young, inexperienced work force with a high turnover that

may be more prone to injury and disease (Burridge et al., 1997, Brown et al., 2011). A large number of workers consumed alcohol regularly and over 10% appeared intoxicated at interview. Alcohol consumption is a risk factor for injury at work (Stallones and Xiang, 2003). Over 20% of the workers smoke and over 20% of workers eat at the slaughterhouse. Smoking and consuming food at work are associated with increased risk of zoonotic disease (Campagnolo et al., 2000). The lack of personal hygiene, lack of hand washing, and slaughtering of sick animals could increase the risk of meat contamination and exposure to disease.

A number of risk factors have been associated with zoonotic disease exposure in slaughterhouse workers that include cutting animals throats (Abu-Elyazeed et al., 1996, Swai and Schoonman, 2009). This practice is performed in ruminant slaughterhouses by a specified slaughterman and in pig only slaughterhouses by the same person that will perform the evisceration. Cleaning animal parts is considered a risk for zoonotic disease exposure (Swai and Schoonman, 2009). Washing the intestines is performed by specific workers in the ruminant slaughterhouse. Workers involved with cleaning intestines do not wear special protective clothing or gloves to reduce their exposure.

Ill workers are a risk to meat contamination and should self report (Mann, 1984). However, as workers are paid per animal slaughtered they are unlikely to take time off if they are feeling sick. A number of workers reported coughs and skin infections within the past 3 months. These conditions can lead to bacterial contamination of meat (Mann, 1984). Workers from ruminant slaughterhouses were more likely to report certain illnesses. The epidemiology of these illnesses warrants further

investigation. Addition research investigating the carriage of *Salmonella* sp., *Shigella* sp., *Escherichia coli* and *Campylobacter* sp. is planned.

A number of workers have a second occupation, predominantly as stakeholders in the meat production industry as farmers or butchers, and are therefore exposed to animals and meat products outside the slaughterhouse. This increased exposure may act as a source of infection or a potential for dissemination since these activities are independently associated with zoonotic disease exposure. These factors will be considered in Chapter 5 which will analyse the risk factors for zoonotic disease exposure in slaughterhouse workers.

3.5 Conclusion

This is the first study of its type in Kenya and the information generated is important to understanding the current situation in the meat industry in western Kenya.

The hypotheses of this study were:

1. slaughterhouses in western Kenya have inadequate infrastructure and poor sanitation
2. slaughterhouse workers in western Kenya have poor hygiene practices
3. slaughterhouse workers are aware of zoonotic diseases

The first hypothesis was proven. The infrastructure at the majority of slaughterhouses did not meet the guidelines with many slaughterhouses lacking basic structural requirements such as a roof and sanitation facilities such as a toilet or running water. The second hypothesis was also proven as many of the workers did not wear protective clothing. However the third hypothesis was not proven as the majority of workers did not know about zoonotic diseases.

The study documents the conditions in slaughterhouses in western Kenya before the implementation of the revised Meat Control Act, 2012 and gives an indication where initial improvements need to be made. None of the slaughterhouses visited complied with the published regulations at the time of the study, with many falling far below a minimum standard.

During the course of the study in 2012, some changes were noticed in slaughterhouses as the new act was brought into effect. The changes were initially focused on ruminant slaughterhouses, which may explain the significant difference

between ruminant and pig slaughterhouses documented in this study through the chi squared analysis. Meat inspectors and District Veterinary Officers informed the study team that each slaughterhouse was urged to adopt one change in the first year or face closure. They were concerned that strictly enforcing the new standards would lead to a deficit in the meat industry in the region. 2012 saw the closure of a number of slaughter facilities which did not make efforts to adopt the changes. The informal meat industry was very difficult to quantify as slaughterhouse owners and butchers were unwilling to admit to slaughtering without authority as they feared prosecution from the public health department. This fear may explain the number of slaughterhouses that declined to participate. Despite only 2 slaughterhouses being classified as category B a large number of slaughterhouses were exporting meat beyond the local village where the slaughter was conducted which contravenes the regulations of the Meat Control Act and may allow the dissemination of disease.

The results of this study are an important contribution to the meat industry in western Kenya and may be generalised to other areas in rural Kenya. Improvements need to be made to facilities and practices in all slaughterhouses. In the initial stages, training is recommended to improve awareness for workers, managers, and inspectors of the risks of meat contamination and methods to reduce it. Secondly, improvement of facilities must be implemented with closure of substandard facilities and focusing resources on fewer facilities to improve meat hygiene in this resource-limited setting.

Chapter 4

Sero-prevalence of brucellosis, leptospirosis, Q fever, Rift Valley fever (RVF), taeniasis and cysticercosis in slaughterhouse workers in western Kenya

4.1 Introduction

Slaughterhouse workers are considered at increased risk of being exposed to zoonotic diseases (Ojo, 1996, McEwen, 1987). This risk is a result of the extremely close contact workers have with a large number of animals and animal products. Furthermore, if meat inspection and public health standards are weak, there may be a higher proportion of sick animals at slaughter as producers sell animals to reduce losses (Brown et al., 2011, Nabukenya et al., 2013). Zoonotic disease outbreaks in slaughterhouse workers in other countries are commonly documented in the literature (Swai and Schoonman, 2009, Wilson et al., 2010, Benschop et al., 2009, Chan et al., 1987, Cousins et al., 1999, Abu-Elyazeed et al., 1996, Whitney et al., 2009). In contrast, the last report in Kenya of zoonoses in slaughterhouse workers was a brucellosis outbreak in 1953 (Wright et al., 1953).

The aim of this component of the study was to assess the seroprevalence of the described pathogens in slaughterhouse workers. Brucellosis, leptospirosis, Q fever, and RVF were chosen because there is a documented risk to slaughterhouse workers (Swai and Schoonman, 2009, Wilson et al., 2010, Chan et al., 1987, Benschop et al., 2009, Abu-Elyazeed et al., 1996, Cousins et al., 1999). There are no published data suggesting that slaughterhouse workers are at increased risk for taeniasis and cysticercosis. It was hypothesised by the author that slaughterhouse workers may have increased exposure to these pathogens through greater access to meat products that are infected. Assessing the seropositivity of slaughterhouse workers through serological testing provides evidence for the potential presence of these organisms in western Kenya.

The hypotheses of this study are that:

1. slaughterhouse workers are exposed to zoonotic disease in western Kenya
2. slaughterhouse workers are more exposed to zoonotic disease than the general population

4.2 Methods

4.2.1 Study design

The study area, population and sampling were described in detail in Sections 2.1, 2.4 and 2.5.

4.2.2 Diagnostic tests

The diagnostic tests used are described in detail in Section 2.7. Sera were tested for brucellosis (Rose Bengal Test), leptospirosis (Panbio Leptospirosis IgM ELISA), Q fever (Serion Classic IgG Phase 2 ELISA), RVF (BDSL cELISA), and cysticercosis (HP10-Antigen ELISA). Faecal samples were tested for taeniasis (Coproantigen ELISA),

4.2.3 Statistical analysis

The apparent prevalence estimates were calculated using the *epi.prev* function in the *EpiR* package (Stevenson, 2014a) of R (R Core Team, 2013). Weights were calculated by dividing the number of expected workers by the number sampled. These were used to calculate a design effect in the *Survey* (Lumley, 2012) package of R. The individual level prevalence results were adjusted with slaughterhouse as the clustering variable using *svymean* in the *Survey* package of R. The true prevalence estimate accounting for the test sensitivity and specificity were calculated using the *truePrev* function in the *prevalence* package (Devleesschauwer et al., 2013) of R. The sensitivity and specificity used in this analysis are highlighted in Table 2.1.

For the commercially available diagnostic kits (leptospirosis, Q fever and RVF) the manufacturer's recommended sensitivity and specificity were used. These values were chosen as there were not any published studies in the region that gave a better

approximation. For the laboratory developed ELISAs (taeniasis and cysticercosis) the sensitivity and specificity used were those reported in similar study sites and published by the laboratories that developed the tests (Fleury et al., 2007, Praet et al., 2013).

For the estimates of the proportion of seropositive slaughterhouses, the data set was collapsed to slaughterhouse level and each slaughterhouse was classified as positive if one or more workers were positive. 95% CIs are given unless otherwise stated.

Data were managed in Microsoft® 2007 Access databases. The ELISA output data for each pathogen is represented graphically using R.

4.2.4 Mapping and spatial cluster analysis

The locations of slaughterhouses were mapped using ArcGIS^{TM} (ESRI, Redlands, California, USA). Ripley's K functions were used in the *spatstat* package (Baddeley, 2005) in R to determine spatial clustering at the slaughterhouse level with slaughterhouses recorded as positive if one or more workers had disease. The methodology is described in detail by Ngowi at al. (Ngowi et al., 2010).

The spatial distribution of slaughterhouses without workers seropositive for the zoonotic disease under examination was considered the control and represented normal heterogeneity. The distribution of the slaughterhouses with one worker seropositive for the zoonotic disease under examination was compared with the control.

The expected number of case slaughterhouses within a fixed distance, 15,000 metres, of a case slaughterhouse was compared to the expected number of control

slaughterhouses. Ripley's K functions were used to assess if the distribution of cases differed from the distribution of controls. The hypothesis was that the K functions would be identical for cases and controls if there was no spatial clustering. This was simulated 1000 times to calculate upper and lower confidence limits. The differences between the K functions for controls and cases were plotted graphically.

Variation from a straight line above zero in the y axis suggests clustering of the samples. Significance is determined if the function crosses the upper 95% CI. If the line falls below zero the clustering is unlikely to be spatial.

4.3 Results

Of the 738 slaughterhouse workers that were recruited and interviewed, 737 consented to giving a blood sample and 729 submitted a faecal sample. All 142 slaughterhouses were represented.

4.3.1 Individual level disease prevalence

Table 4.1 shows the individual level prevalence estimates (apparent prevalence) from the tests. The apparent prevalence of leptospirosis was 13.4% and that of Q fever was 4.5%. The prevalence for RVF, cysticercosis and *Taenia* were much lower: 1.2%, 2.6% and 1.7% respectively and only 0.1% for brucellosis. Table 4.1 also shows the adjusted prevalence estimates accounting for the design effect. There was not a marked difference in the prevalence estimates after this adjustment.

The true prevalence after adjustment for the sensitivity and specificity of the tests is shown in Table 4.1. There was not a large difference for brucellosis, leptospirosis, Q fever or RVF. However the true prevalence for taeniasis and cysticercosis were markedly changed.

Disease	Apparent prevalence (95% CI) n=737	Adjusted prevalence for design effect (95% CI)	Adjusted prevalence for se/sp† (95% CI)
Brucellosis	0.1 (0.007–0.8)	-	0.3 (0–0.8)
Leptospirosis	13.4 (11.1–16.1)	13.6 (10.9–16.4)	12.7 (10.2–15.4)
Q fever	4.5 (3.2–6.2)	4.6 (3.1–6.1)	3.4 (1.9–5.1)
RVF	1.2 (0.6–2.3)	1.2 (0.4–2.1)	1 (0.3–2.0)
Taenia* (n=691)	1.9 (1.1–3.2)	1.7 (0.8–2.5)	0.2 (0–1.0)
Cysticercosis	2.6 (1.7–4.0)	2.8 (1.5–4.1)	0.3 (0–0.9)

*Only 691 faecal samples were sufficient in volume for the coproantigen ELISA.
† se/sp sensitivity/specificity

Table 4.1 Individual level prevalence estimates for 6 zoonoses in workers

4.3.2 Brucellosis

There was only one individual positive for brucellosis. Therefore it was not possible to account for the design affect. It was also not possible to look at the difference in prevalence between slaughterhouse types.

4.3.3 Leptospirosis

Table 4.2 shows the leptospirosis prevalence for workers in different slaughterhouse types corrected for the design effect and the test sensitivity and specificity. The apparent prevalence does not differ between individuals in the different slaughterhouse types. The apparent prevalence of leptospirosis in mixed ruminant slaughterhouse workers is 13.5% while in both cattle only slaughterhouse workers and pig only slaughterhouse workers it is 13.4%. The apparent prevalence did not change markedly after accounting for the design effect but did change when the test sensitivity and specificity were considered.

Figure 4.1 are histograms of the Panbio units from the leptospirosis ELISA for each slaughterhouse type. The red line indicates the negative cut-off and the blue line the positive cut-off. Equivocal results (between the lines) were considered negative for the purposes of this study. The distribution of positives is similar across the slaughterhouse types.

Figure 4.2 shows the location of the slaughterhouses and the number of leptospirosis positive workers. The leptospirosis positive slaughterhouses were distributed evenly throughout the study site with a higher number in the middle of the study area. There was no statistical evidence of spatial clustering of positive slaughterhouses (Appendix 6).

	Mixed ruminant n=274 (95% CI)	Cattle only n=292 (95% CI)	Pig only n=171 (95% CI)
Individual level apparent prevalence (n=737)	13.5 (10.0–18.0)	13.4 (9.9–17.7)	13.4 (8.0–18.7)
Individual result adjusted for the design effect (n=737)	13.7 (8.9–18.5)	13.3 (9.4–17.3)	13.9 (8.1–19.7)
Individual result adjusted for test se/sp† (n =737)	12.9 (8.9–17.5)	12.8 (8.7–17.1)	13.0 (8.1–18.7)

† se/sp sensitivity/specificity

Table 4.2 Leptospirosis seroprevalence estimates for slaughterhouse workers

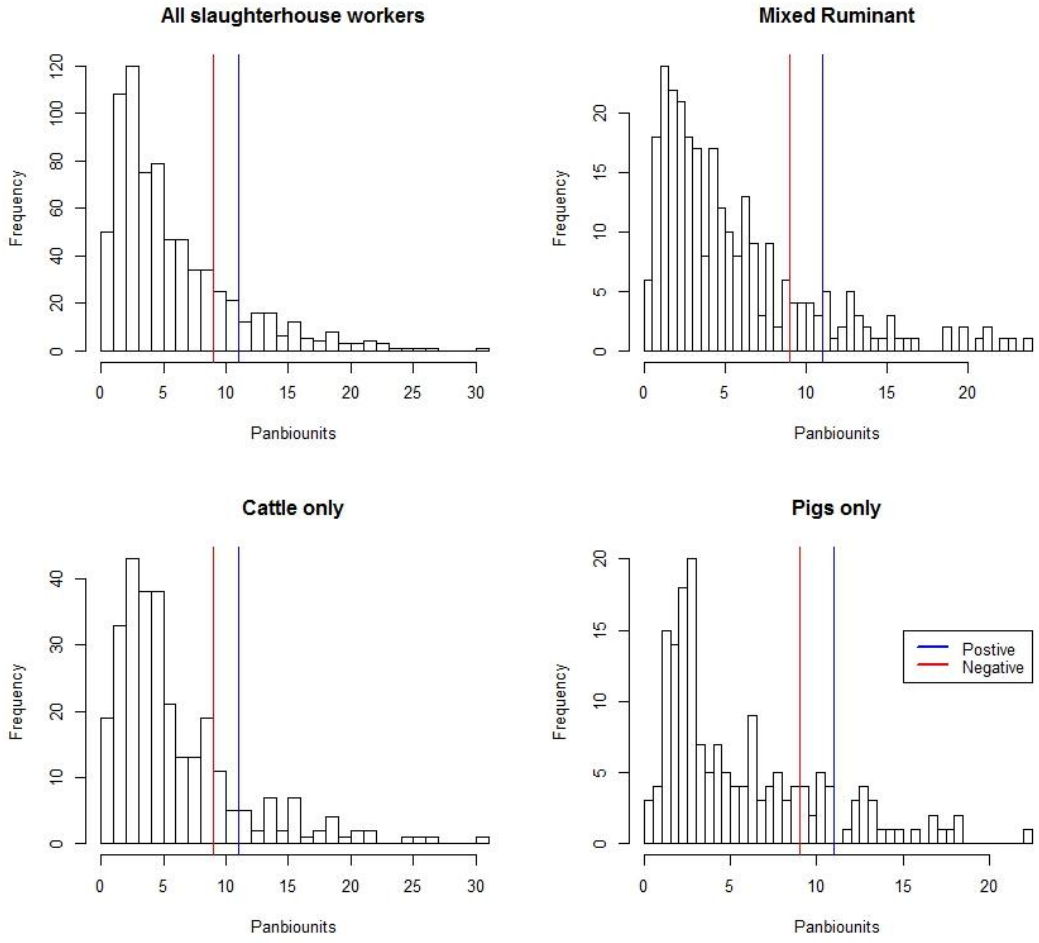
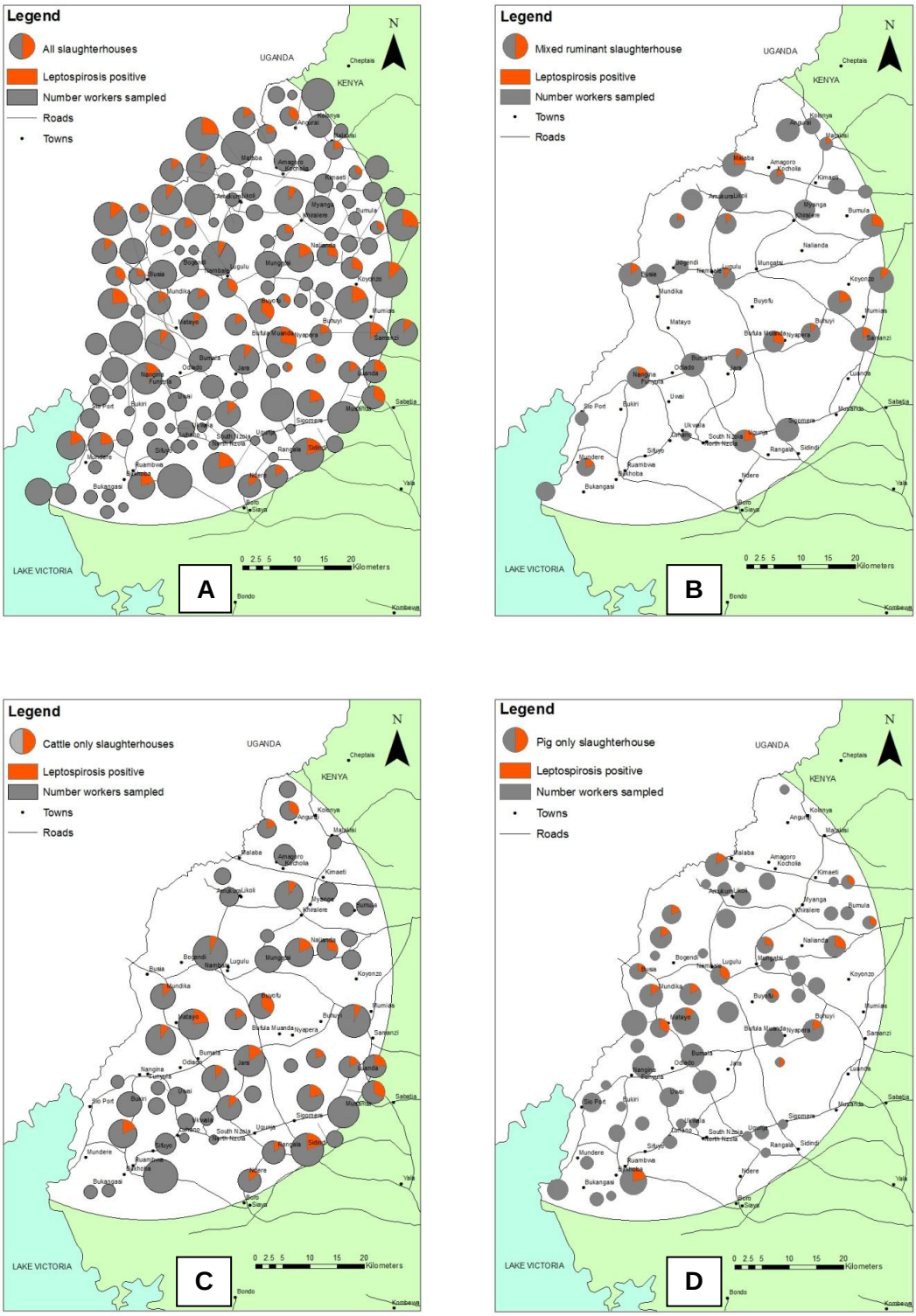


Figure 4.1 Histogram of Panbio leptospirosis ELISA Panbio units



A) All slaughterhouses, B) Mixed Ruminant, C) Cattle only, D) Pig only

Figure 4.2 Map of leptospirosis positive slaughterhouses

4.3.3 Q fever

Table 4.3 shows the Q fever prevalence in different slaughterhouse types corrected for the design effect and the test sensitivity and specificity. The apparent prevalence was highest in mixed ruminant slaughterhouse workers (6.2%), moderate in cattle only slaughterhouse workers (4.1%), and lowest in pig only slaughterhouses workers (2.3%). The prevalence was not markedly affect by the design but changed when the sensitivity and specificity of the test was considered.

Figure 4.3 demonstrates histograms of the corrected OD values from the Q fever ELISA for each slaughterhouse type. The red line is the negative cut-off and the blue line is the positive cut-off. Values in between were considered negative for the purposes of this study. The histograms demonstrate a small positive population with positives having markedly high OD readings in some cases.

Figure 4.4 shows the location of the slaughterhouses and the number of Q fever positive workers. The Q fever positive slaughterhouses are distributed throughout the study site although there is an absence of Q fever positive slaughterhouses in the southern part of the study area. There was no statistical evidence of spatial clustering of positive slaughterhouses (Appendix 6).

	Mixed ruminant n=274 (95% CI)	Cattle only n=292 (95% CI)	Pig only n=171 (95% CI)
Individual level apparent prevalence (n=737)	6.2 (3.9–9.7)	4.1 (2.4–7.0)	2.3 (0.9–5.8)
Individual result adjusted for the design effect (n=737)	6 (3.3–8.8)	4.3(1.9–6.6)	2.4 (0.4–4.5)
Individual result adjusted for test se/sp† (n =737)	5.4 (2.6–8.9)	3.2 (1.0–5.9)	1.8 (0.1–4.9)

† se/sp sensitivity/specificity

Table 4.3 Q fever seroprevalence estimates for slaughterhouse workers

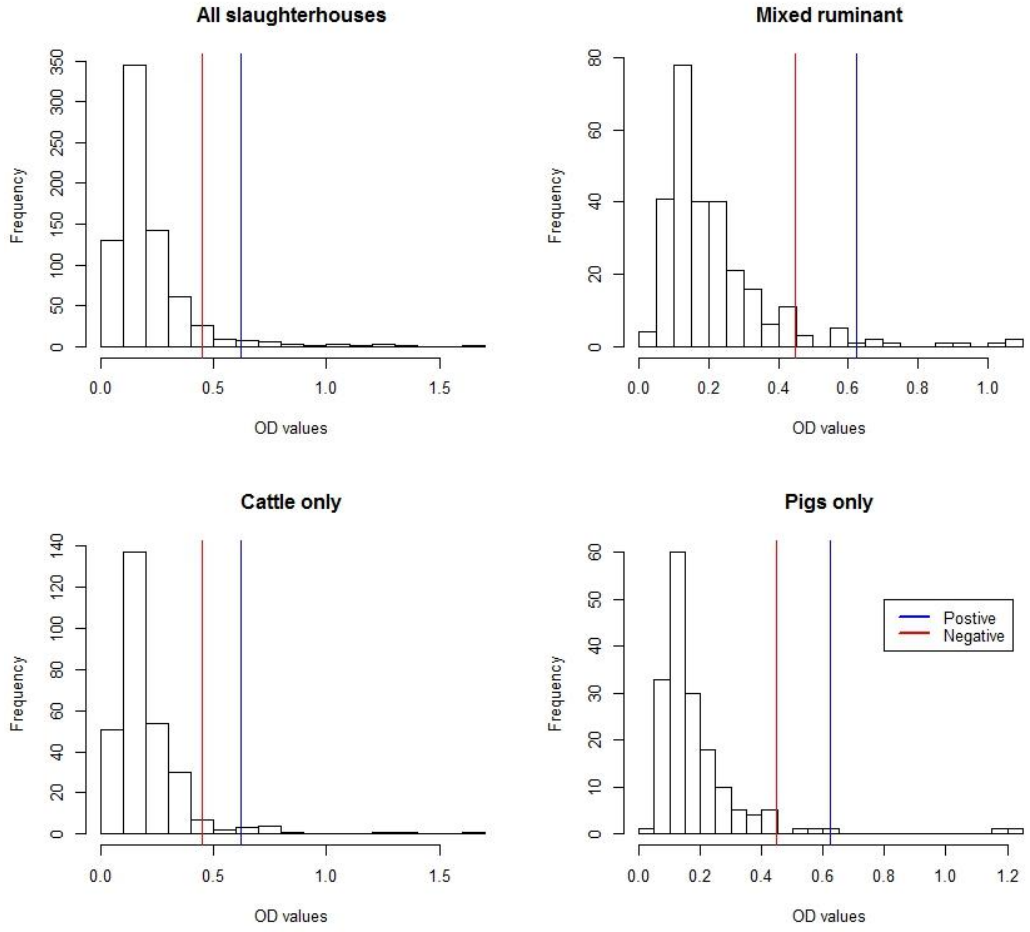
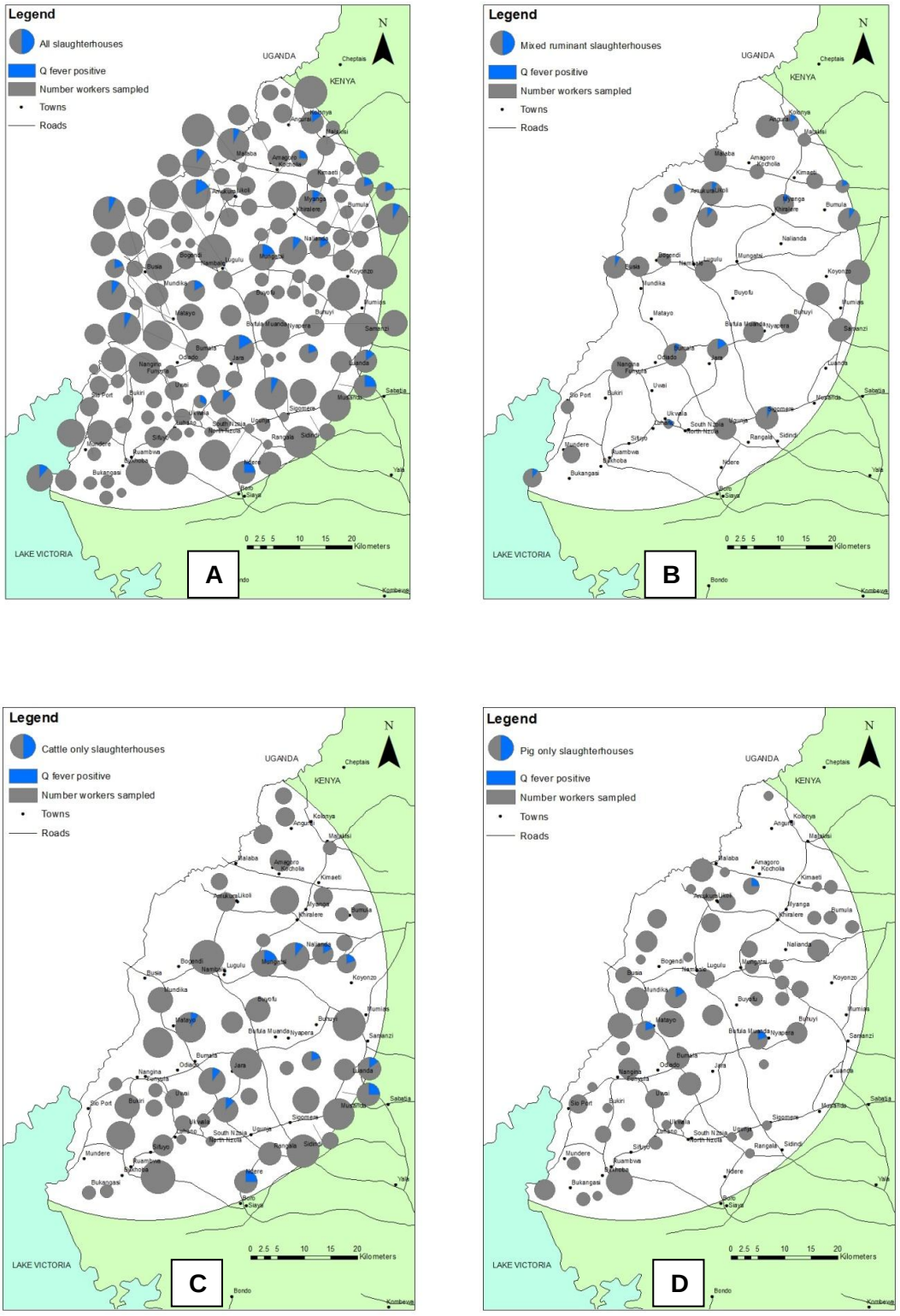


Figure 4.3 Histogram of Q fever optical density (OD) values for slaughterhouse workers



A) All slaughterhouses, B) Mixed ruminant, C) Cattle only, D) Pig only

Figure 4.4 Map of Q fever positive slaughterhouses

4.3.4 RVF

Table 4.4 shows the RVF prevalence in different slaughterhouse types corrected for the design effect and the test sensitivity and specificity. The apparent prevalence estimates were very low and not markedly different between slaughterhouse types. The apparent prevalence estimates were 1.1% of mixed ruminant slaughterhouse workers, 1.4% for cattle only slaughterhouse workers and 1.2% for pig only slaughterhouse workers. Neither the design effect nor the test altered the apparent prevalence markedly.

Figure 4.5 demonstrates histograms of the PI units from the RVF ELISA for each slaughterhouse type. The histograms show a very small positive population with positive results having very high PI values.

Figure 4.6 shows the location of the slaughterhouses and the number of RVF positive workers. There was no statistical evidence of spatial clustering of positive slaughterhouses (Appendix 6).

	Mixed ruminant n=274 (95% CI)	**Cattle only n=292 (95% CI)**	**Pig only n=171 (95% CI)**
Individual level apparent prevalence (n=737)	1.1 (0.3–3.2)	1.4 (0.5–3.5)	1.2 (0.3–4.1)
Individual result adjusted for the design effect (n=737)	1.4 (0–3.0)	1.2 (0–2.5)	1.0 (0–2.3)
Individual result adjusted for test se/sp† (n =737)	1.2 (0.1–2.9)	1.4 (0.2–3.1)	1.4 (0.1–3.8)

† se/sp sensitivity/specificity

Table 4.4 Rift Valley fever seroprevalence estimates for slaughterhouse workers

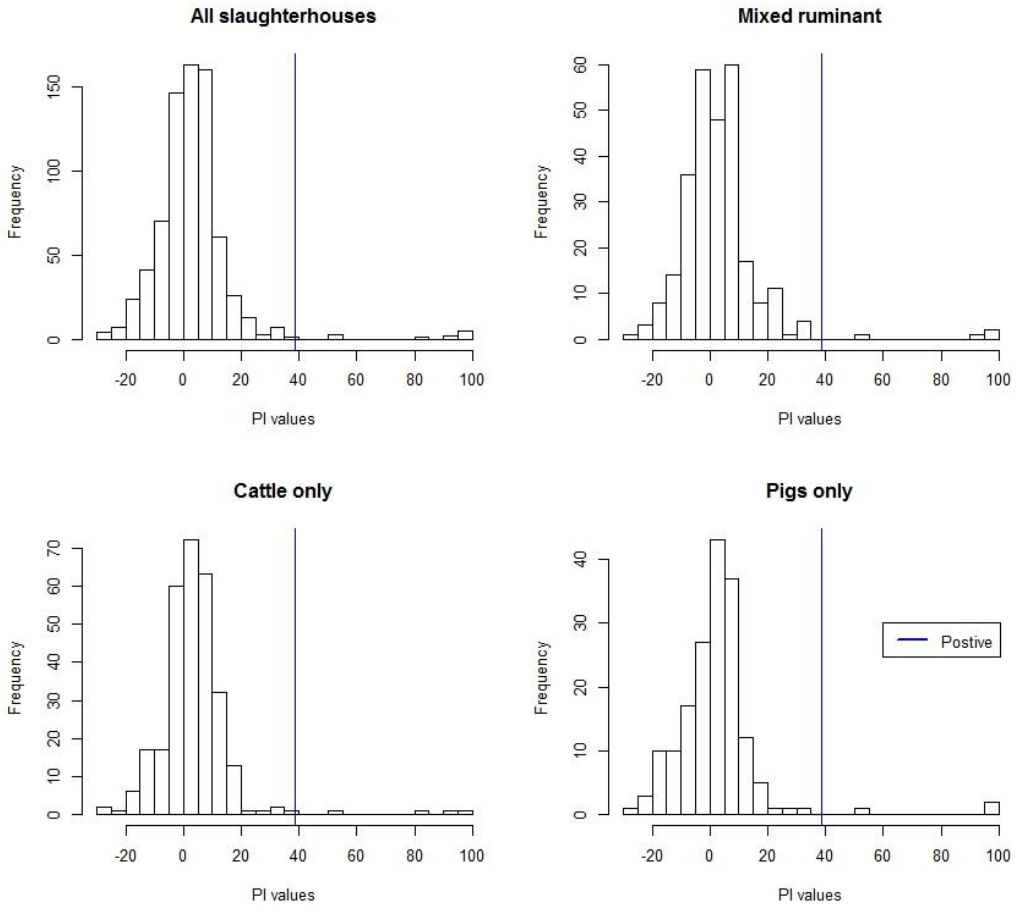
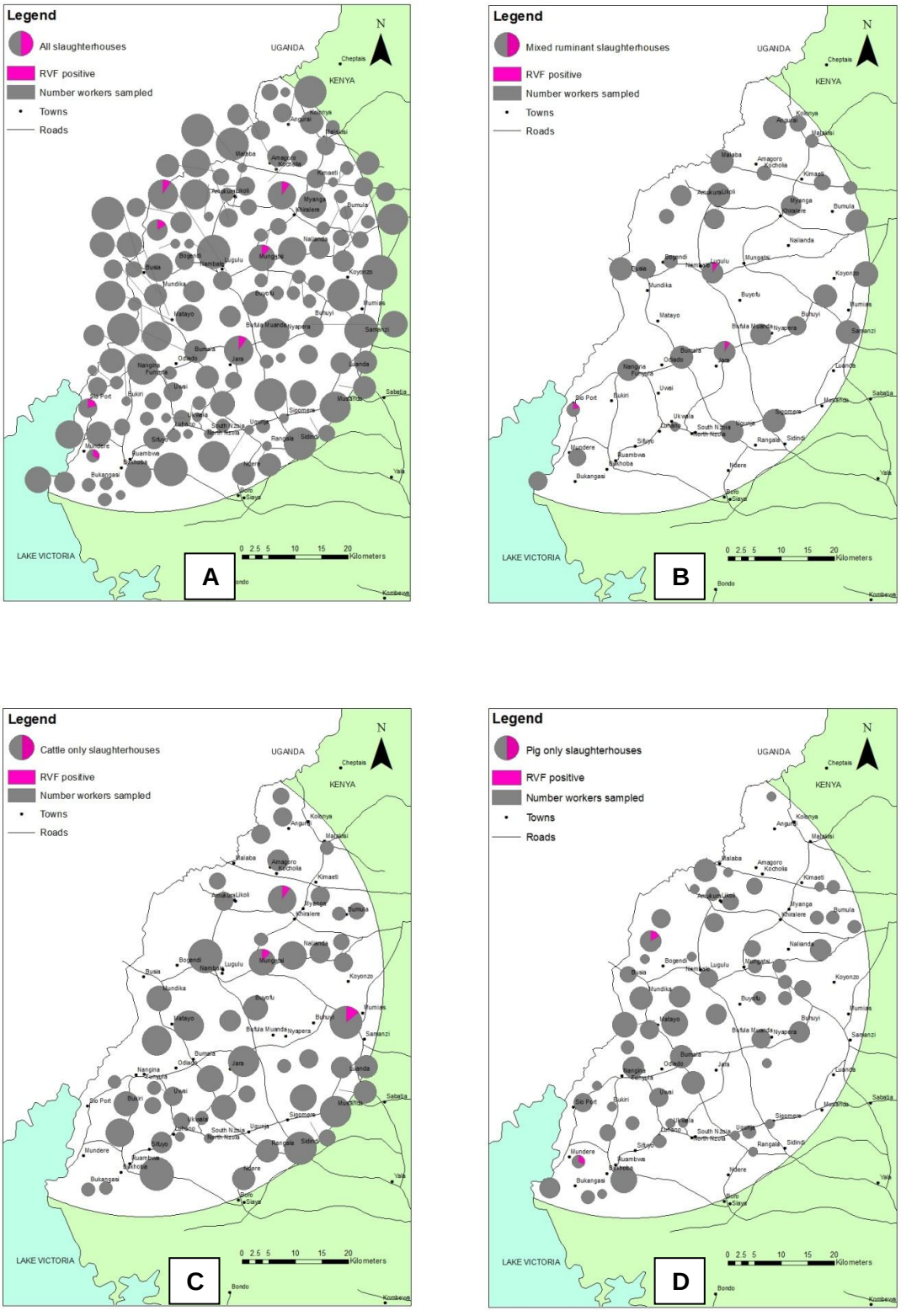


Figure 4.5 Histogram of RVF percentage inhibition (PI) values



All slaughterhouses, B) Mixed ruminant, C) Cattle only, D) Pig only

Figure 4.6 Map of Rift Valley fever positive slaughterhouses

4.3.5 *Taenia*

Table 4.5 shows the *Taenia* prevalence in different slaughterhouse types corrected for the design effect and the test sensitivity and specificity. The greatest apparent prevalence was recorded in workers from cattle only slaughterhouses (3.7%), followed by pig only slaughterhouses (1.3%). The lowest prevalence was in mixed ruminant slaughterhouse workers (0.4%). The design effect did not alter the prevalence. However, accounting for the test sensitivity and specificity markedly altered the individual prevalence in cattle only slaughterhouses from 3.7% to 0.7%. The true prevalence of *Taenia* sp. in cattle only slaughterhouse workers (0.7%) is similar to mixed ruminant slaughterhouse workers (0.5%) and pig only slaughterhouse workers (0.9%).

Figure 4.7 demonstrates the OD values from the coproantigen ELISA. The blue line indicates the positive cut-off. The histograms for each slaughterhouse show a very small positive group with high OD values.

Figure 4.8 shows the location of the slaughterhouses and the number of *Taenia* positive workers. There appears to be an absence of *Taenia* positive slaughterhouses in the northern part of the study area. However, there was no statistical evidence of spatial clustering of positive slaughterhouses (Appendix 6).

	Mixed ruminant n=261 (95% CI)	Cattle only n=273 (95% CI)	Pig only n=157 (95% CI)
Individual level apparent prevalence (n=691)	0.4 (0.01–2.1)	3.7 (2.0–6.6)	1.3 (0.4–4.5)
Individual result adjusted for the design effect (n=691)	0.4 (0–1.1)	3.7 (1.8–5.7)	1 (0–2.2)
Individual result adjusted for test se/sp† (n =691)	0.5 (0–1.8)	0.7 (0–2.6)	0.9 (0–3.1)

† se/sp sensitivity/specificity

Table 4.5 *Taenia* prevalence estimates for slaughterhouse workers

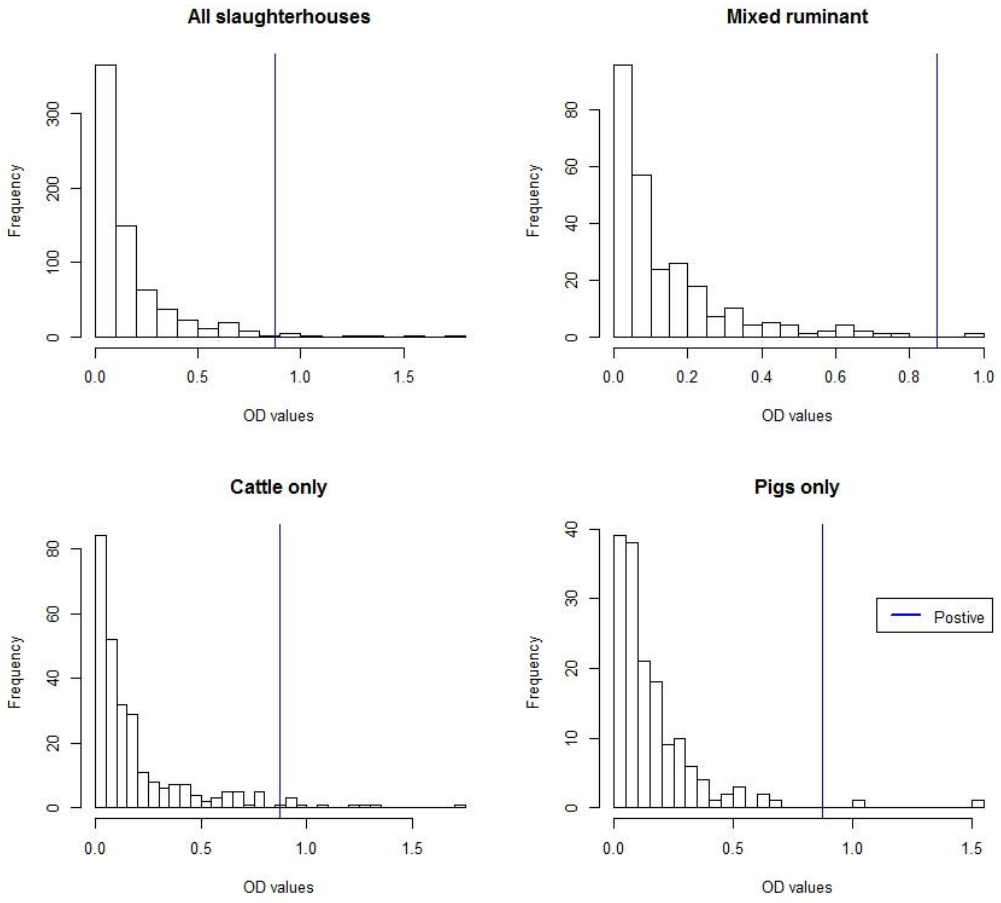
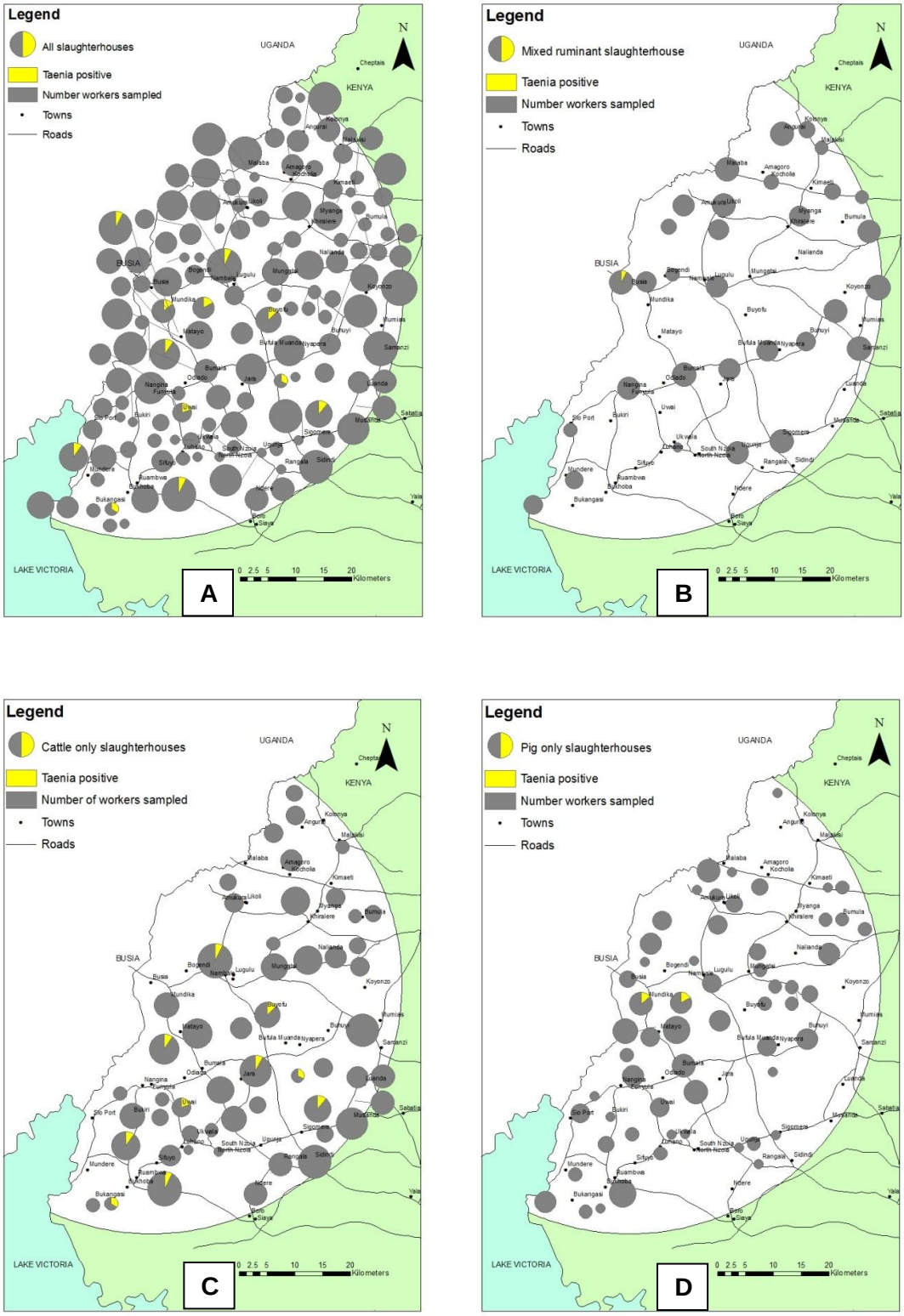


Figure 4.7 Histogram of coproantigen ELISA optical density (OD) values for slaughterhouse workers



A) All slaughterhouses, B) Mixed ruminant, C) Cattle only, D) Pig only

Figure 4.8 Map of taeniasis positive slaughterhouses

4.3.6 Cysticercosis

Table 4.6 shows the cysticercosis prevalence in different slaughterhouse types corrected for the design effect and the test sensitivity and specificity. The greatest apparent seroprevalence was in pig only slaughterhouse workers (4.1%). Mixed ruminant slaughterhouse workers had a seroprevalence of 2.6% and cattle only slaughterhouse workers a seroprevalence of 1.7%. The apparent seroprevalence was not markedly changed by the design effect. Accounting for the test imperfections did change the seroprevalence estimates. Pig only slaughterhouse workers still had the greatest true seroprevalence (1.4%).

Figure 4.9 demonstrates the OD values from the HP10 ELISA. The histograms for each slaughterhouse type show a small positive population with moderately high OD values.

Figure 4.10 shows the location of the slaughterhouses and the number of cysticercosis positive workers. There was no statistical evidence of spatial clustering of positive slaughterhouses (Appendix 6).

	Mixed ruminant n=274 (95% CI)	Cattle only n=292 (95% CI)	Pig only n=171 (95% CI)
Individual level apparent prevalence (n=737)	2.6 (1.2–5.2)	1.7 (0.7–3.9)	4.1 (2.0–8.2)
Individual result adjusted for design effect (n=737)	3 (0.5–5.6)	1.8 (0.4–3.2)	4.2 (1.6–6.8)
Individual result adjusted for test se/sp† (n =737)	0.7 (0–2.3)	0.5 (0–1.8)	1.4 (0–4.7)

† se/sp sensitivity/specificity

Table 4.6 Cysticercosis seroprevalence estimates for slaughterhouse workers

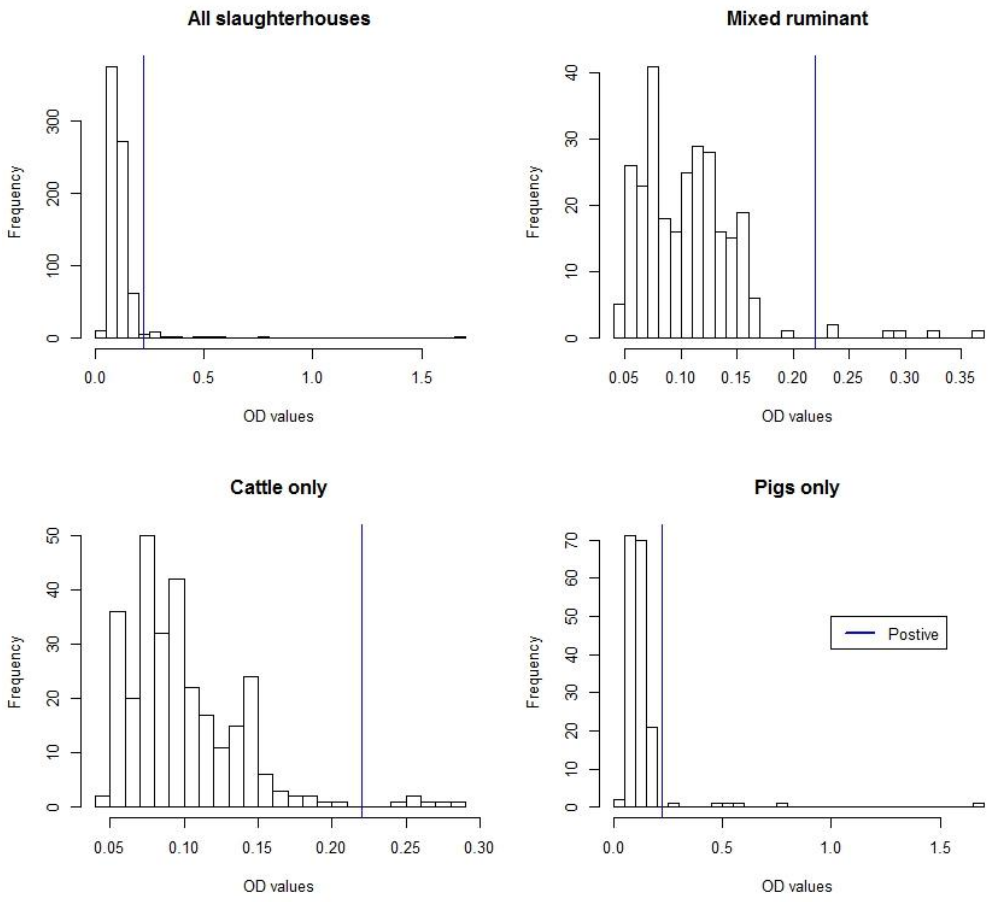
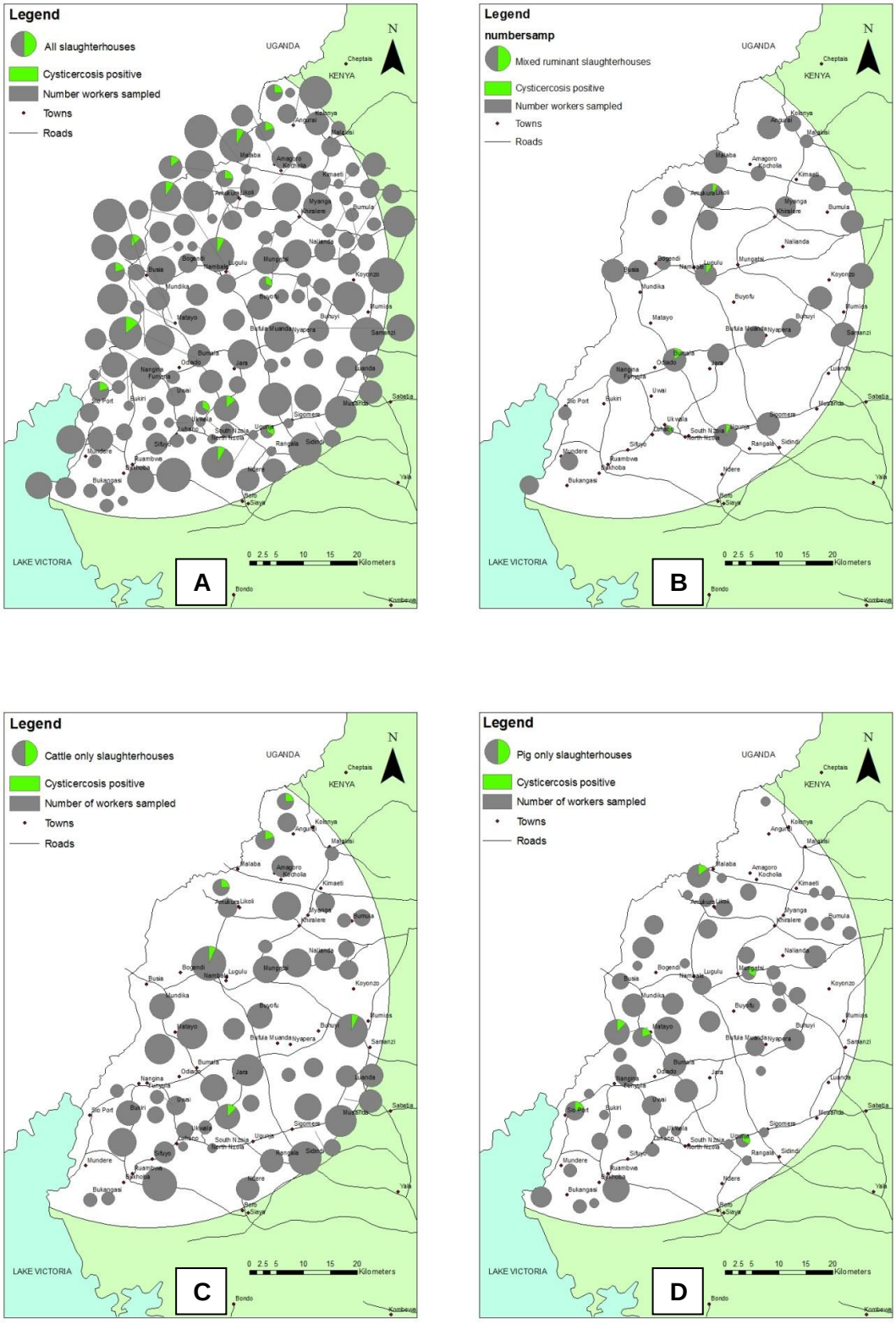


Figure 4.9 Histogram of HP10 ELISA optical density (OD) values for slaughterhouse workers



A) All slaughterhouses, B) Mixed ruminant, C) Cattle only, D) Pig only

Figure 4.10 Map of cysticercosis positive slaughterhouses

4.3.7 Slaughterhouse level disease prevalence

Table 4.7 shows the overall slaughterhouse level prevalence estimates. These data were calculated for slaughterhouses that had at least one positive worker. The apparent slaughterhouse level prevalence was 40.8% for leptospirosis and 19.7% for Q fever. The apparent slaughterhouse level prevalence for RVF, cysticercosis, and *Taenia* were lower: 5.6%, 12%, and 9.1% respectively. The apparent slaughterhouse level seroprevalence for brucellosis was 0.7%.

Disease	Apparent prevalence (95% CI) n=142
Brucellosis	0.7 (0.04–0.9)
Leptospirosis	40.8 (33.1–49.1)
Q fever	19.7 (14.0–27.0)
RVF	5.6 (2.9–10.7)
Taenia	9.1 (5.4–15.0)
Cysticercosis	12.0 (7.6–18.3)

Table 4.7 Slaughterhouse level apparent and adjusted prevalence to 6 zoonoses

Table 4.8 indicates the slaughterhouse level apparent prevalence of each disease for the 3 slaughterhouse types. Mixed ruminant slaughterhouses had higher apparent prevalence estimates than cattle only and pig only slaughterhouses for all diseases except taeniasis. At the slaughterhouse level there is marked difference between prevalence levels for taeniasis among slaughterhouse types. The apparent prevalence is greatest in cattle only slaughterhouses (17%).

	Mixed ruminant n=31 (95% CI)	Cattle only n=53 (95% CI)	Pig only n=58 (95% CI)
Leptospirosis	54.8 (37.8–708)	45.3 (32.7–58.5)	29.3 (19.2–42.0)
Q fever	41.9 (26.4–59.2)	20.8 (12.0–33.5)	6.9 (2.7–16.4)
RVF	9.7 (3.3–24.9)	5.7 (1.9–15.4)	3.1 (0.6–11.5)
Taenia	3.2 (0.2–16.2)	17.0 (9.2–29.2)	3.4 (1.0–11.7)
Cysticercosis	16.1 (7.1–32.6)	11.3 (5.3–22.6)	10.3 (4.8–20.8)

Table 4.8 Slaughterhouse level prevalence of zoonoses in the 3 slaughterhouse types

Figure 4.11 demonstrates the disease state of all slaughterhouses. These data are presented as an indication of disease presence in the slaughterhouse and are not proportional to the number of workers affected. The size of the pie is proportional to the number of workers in the slaughterhouse.

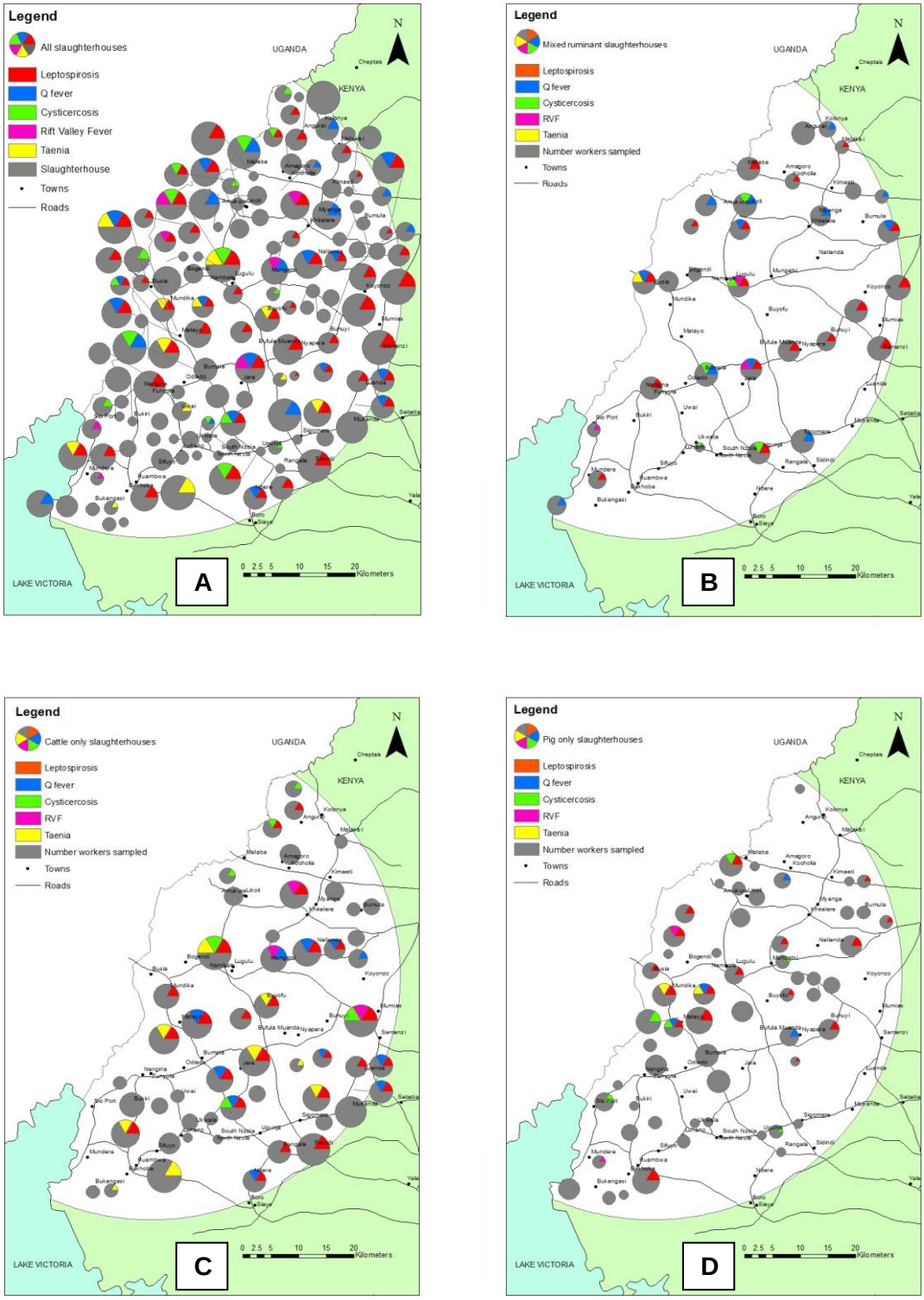


Figure 4.11 Map showing slaughterhouses positive for zoonoses

4.4 Discussion

The seroprevalence results are presented as apparent prevalence estimates and adjusted for design and test imperfections. It appears from this study that the design effect is largely unimportant as the clustered sampling design had little effect on seroprevalence estimates. The effect of the test sensitivity and specificity was more important. The test results for each disease will be discussed separately.

4.4.1 Brucellosis

There is almost a complete absence of brucellosis in slaughterhouse workers in the study area as only one individual (0.1%) was positive on the RBT. This result is in marked contrast to studies from neighbouring countries Uganda, Tanzania and Sudan, where brucellosis has been reported in slaughterhouse workers with seroprevalence rates respectively of 10%, 5.5% and 9% (Nabukenya et al., 2013, Swai and Schoonman, 2009, Omer et al., 2010).

Infection in people is related to carriage of the organism in animals and Kenya is an endemic region for brucellosis in livestock (McDermott and Arimi, 2002, Pappas et al., 2006). The absence of brucellosis in slaughterhouse workers in western Kenya is likely due to the nature of the farming system and low prevalence rates in animals in the region. There is a wide range of brucellosis seropositivity across different regions in Kenya. Animals grazed in large herds on community land are more likely to be seropositive than those in smaller herds (Kadohira et al., 1997). The production system in western Kenya is small farms with small herds which may not maintain the organism (Muma et al., 2012, Matope et al., 2010). This result is supported by the

findings of the PAZ study which reported prevalence of 0.4% in cattle and 0.6% in people (de Glanville, 2014).

The brucellosis testing was performed using the RBT. The advantage of the RBT is that it is easy to run and comparatively cheap and this study was an opportunity to assess the performance of the RBT in a Kenyan setting (Diaz et al., 2011). The RBT has been shown to be a good test for diagnosis of acute cases but performs less well for chronic or complicated cases (Araj et al., 1988). It has been suggested that ELISAs are better for seroprevalence studies (Araj et al., 1986). Diagnostic tests used for diagnosing brucellosis are developed for diagnosis of clinical disease, and since no individual test is perfect, tests are usually used in combination to confirm diagnosis (Yohannes et al., 2012). In future prevalence studies it might be worth considering duplicate testing.

4.4.2 Leptospirosis

There was a high apparent seroprevalence (13.4%) of leptospirosis in slaughterhouse workers. Leptospirosis is commonly reported in slaughterhouse workers in many regions and a study in neighbouring Tanzania reported slaughterhouse workers to have a leptospirosis seroprevalence of 17.1%, which is similar to the findings of this study (Schoonman and Swai, 2009, Benschop et al., 2009).

The difference in seroprevalence between workers at the three slaughterhouse types was minimal (mixed ruminants 13.5%, cattle only 13.4%, and pig only 13.4%). This suggests that the individual risk for exposure to leptospirosis is not dependant on the type of animal slaughtered.

There was a higher prevalence of leptospirosis in mixed ruminant slaughterhouses (54.8%) than the other slaughterhouse types when considering the slaughterhouse as a single unit. This result may be related to slaughterhouse size. It was shown in Chapter 3 that mixed ruminant slaughterhouses have more workers and slaughter a greater number of animals than the other slaughterhouse types. The higher passage of animals through the slaughterhouse may explain the increased prevalence of disease in this subgroup. However it was also shown in Chapter 3 that mixed ruminant slaughterhouses have improved facilities and practices in relation to the other slaughterhouse types. In theory, the improved facilities and practices should lead to a lower prevalence. There may be other unexplored factors that contribute to the increased prevalence of disease in this subgroup of slaughterhouses. The risk factors for leptospirosis in slaughterhouse workers will be examined in the next chapter.

There did not appear to be any geographical clustering of the positive slaughterhouses. There is often spatial clustering of leptospirosis associated with a point source infection, such as an infected water source (Barcellos and Sabroza, 2001, Soares et al., 2010). This situation does not appear to be the case with slaughterhouse workers in western Kenya. The findings of this study are consistent with workers being occupationally exposed rather than exposed to a contaminated water source.

The seroprevalence in the PAZ community for leptospirosis was 4.8%, which is markedly lower than the prevalence in slaughterhouse workers of 13.4%. These data suggest that slaughterhouse workers are more exposed to leptospirosis. This result will be explored further in Chapter 7.

This study used a commercial IgM ELISA on a single serum sample to determine seropositivity. Although the MAT is considered the gold standard for leptospirosis diagnosis, the complexity of the test limits its use to reference laboratories, so commercial IgM ELISAs are used commonly in resource poor settings (Budihal and Perwez, 2014, Adler et al., 1980). Sensitivity of the ELISA compared with MAT is generally good overall, although there is the possibility of regional variation, and although the ELISA detects antibodies to a range *Leptospira*, it does not distinguish between serovars (Bajani et al., 2003, Winslow et al., 1997, Adler et al., 1980).

The performance of the IgM ELISA has yet to be determined in a Kenyan setting. It is impossible to determine without reference to a "gold standard" the performance of the ELISA in this region. The ELISA was developed to detect antibodies to a wide range of leptospiral antigens. However, it is possible that the ELISA may not detect the serovars common in this environment which would affect the sensitivity of this test (Effler et al., 2002). The distribution of the Panbio units for the Panbio leptospira IgM ELISA did not demonstrate a clear distinction between the negative and positive results using the manufacturer's cut-off. There were a large number of equivocal results. Other studies in endemic tropical areas have raised concerns over the use of the Panbio leptospirosis ELISA for diagnosis of clinical leptospirosis in these settings. People in these regions are likely to have persistent antibodies to leptospirosis that will impair the accuracy of the test in diagnosing acute cases (Desakorn et al., 2012, Blacksell et al., 2006). Although clinical diagnosis is not an issue for this study, previously exposed individuals may explain the large number of "equivocal" results.

4.4.3 Q fever

The apparent seroprevalence for Q fever in slaughterhouse workers was 4.5%. Published reports of Q fever in slaughterhouse workers have reported seroprevalence estimates ranging from 12–90% (Cetinkaya et al., 2000, Perez-Trallero et al., 1995, Riemann et al., 1975, Van Peenen et al., 1978). It is difficult to compare seroprevalence reports across regions because different tests are used (Blaauw et al., 2012).

Q fever apparent seroprevalence was higher in workers in mixed ruminant (6.2%) and cattle only slaughterhouses (4.1%) than pig only slaughterhouses (2.3%). These data are consistent with the source of infection being animals at the ruminant (cattle, goats, sheep) slaughterhouses (Raoult and Marrie, 1995). Working in a pig slaughterhouse is not a known risk factor for Q fever (Australian Government, 2013). The apparent prevalence at the slaughterhouse level is markedly higher in mixed ruminant slaughterhouses, which further support the hypothesis that workers are exposed to infected animals (cattle, goats, sheep) at work. The risk factors for Q fever exposure will be investigated further in Chapter 5.

The adjusted Q fever seroprevalence in the PAZ community was 2.2% (de Glanville, 2014) compared to 4.5% in slaughterhouse workers. This disparity suggests that slaughterhouse workers are more exposed to Q fever than the general population. This result will be explored further in Chapter 7.

There was no apparent geographical clustering of Q fever positive slaughterhouses, which again points to exposure at the slaughterhouse and not another point source.

Point source outbreaks of Q fever have been associated with proximity to goat farms (Roest et al., 2011)

The results of the Serion Classic *C. burnetii* IgG Phase 2 ELISA OD show a large negative population and a small positive population. The ELISA used in this study has good sensitivity and specificity for detection of Q fever Phase 2 antibodies and is recommended for serological surveillance (Peter et al., 1987). However, as in all cases it is possible that during convalescence antibodies fall below the cut off (Blaauw et al., 2012). Antibodies also fall after treatment (Peter et al., 1988). The treatment for Q fever is doxycycline. In Kenya tetracyclines (including doxycycline) are the second most dispensed antibiotics (Global Antibiotic Resistance Partnership, 2011) so even if misdiagnosed, it is possible that Q fever patients may receive effective treatment. This may result in an underestimation of the number of Q fever positive cases in the population.

4.4.4 RVF

The apparent seroprevalence for RVF in the slaughterhouse workers was 1.2%. The numbers of seropositive workers for RVF initially seem quite low but these results are comparable to other studies that have been conducted in RVF endemic areas. For example, in Egypt and Saudi Arabia, seroprevalence to RVF in slaughterhouse workers was 2% and 0.72% respectively (Abu-Elyazeed et al., 1996, Azhar et al., 2010).

There was no difference in apparent seroprevalence between the slaughterhouse types (Table 4.4). These data suggest that exposure may not occur in the slaughterhouse. Furthermore, as the virus is predominantly carried by ruminants

there should be an increased prevalence in mixed ruminant and cattle only slaughterhouses. This distinction is not documented in this study.

RVF virus has never been described before in western Kenya. This study reports a number of people in western Kenya to be historically exposed to RVF but it cannot make conclusions about where the individuals encountered the virus. There is no obvious geographic relationship between positive cases. It is possible that the seropositive workers were affected with RVF outside the study area. Further investigation of RVF seropositivity in the community was conducted as part of the PAZ study and the apparent prevalence was 1.5%. This prevalence is similar to the findings in slaughterhouse workers, suggesting that slaughterhouse workers are not more exposed than the community.

In relation to other areas in Kenya where RVF is considered enzootic, studies have shown the inter-epidemic seroprevalence to be between 1 and 19% (LaBeaud et al., 2007). Antibodies to RVF are likely to be life-long so the presence of IgG antibodies is indicative of historical exposure (Findlay and Howard, 1951). This is particularly valuable information for serological surveys in areas where seroprevalence is low (LaBeaud et al., 2007). This result suggests that it is epidemiologically plausible that RVF virus is circulating in western Kenya.

In regard to the BDSL RVF cELISA it is a technically demanding test and required extensive optimisation in our laboratory. It is not a suitable test for a one-off study of seroprevalence.

4.4.5 *Taenia*

The prevalence estimates for taeniasis (1.8%) are lower than previous reports of taeniasis in the region. A previous study found the carriage of *Taenia* sp. eggs by faecal examination to be 3.2% (Kagira et al., 2011). The PAZ study reported a prevalence of 19.7% in the community using the coproantigen ELISA (de Glanville, 2014). These results suggest that slaughterhouse workers are not at particular risk of exposure to *Taenia* sp.

The route of exposure to *Taenia* is different to the other pathogens since the intermediate stage is ingested in meat. The prevalence of bovine cysticercosis is high in Kenya which means transmission risk is high (Onyango-Abuje et al., 1996). It is possible that slaughterhouse workers are more selective about the meat they eat as they can see the lesions at slaughter or they cook the meat well enough to destroy the infective larvae.

The apparent prevalence is greatest in cattle slaughterhouses suggesting infection with *T. saginata* over *T. solium*. However when accounting for the test sensitivity and specificity, the true prevalence in cattle only slaughterhouse workers is the similar to that of mixed ruminant and pig only slaughterhouses. This similarity suggests a large number of false positives in the original results. Copro PCR has improved specificity and should be considered as an alternative test (Praet et al., 2013).

There was no apparent clustering of slaughterhouses that had taeniasis positive workers, which is consistent with the PAZ findings that human taeniasis in the community is not spatially clustered (Thomas, 2013).

At the slaughterhouse level, the apparent prevalence for taeniasis is greatest in cattle only slaughterhouses. This result did not take into account the test sensitivity or specificity. A hierarchical Bayesian model would be required to calculate the true prevalence which was beyond the scope of this thesis (Scolamacchia et al., 2010).

The coproantigen ELISA is twice as effective in detecting cases of taeniasis compared to microscopy (Allan and Craig, 2006). However, the sensitivity and specificity of the ELISA is still relatively poor. It is important to consider that at such a low prevalence this test is likely to have low positive predictive value and hence reduce confidence in the results.

4.4.6 Cysticercosis

The seroprevalence of cysticercosis in slaughterhouse workers was 2.6%. The results are lower than expected in this population. Other studies in this area found prevalence up to 10% in the region (Mafojane et al., 2003). This discrepancy may be related to the age and/or gender of the population, as seroprevalence rates are greater in women and children than adult men (Mafojane et al., 2003, Mwanjali et al., 2013). The prevalence in the PAZ adjusted population was 6.6% but, when adjusted for the study design and test sensitivity and specificity, the reported prevalence was 0.8% (Thomas, 2013). This result is similar to the findings of cysticercosis in slaughterhouse workers. Exposure to cysticercosis is not related to working in the slaughterhouse, as transmission requires contact with a *T. solium* tapeworm carrier. This study suggests that working in the slaughterhouse is not a risk for cysticercosis. This result is supported by other studies that have shown that slaughtering is not a significant risk factor for cysticercosis (Sikasunge et al., 2007).

The greatest seroprevalence was found in workers at pig only slaughterhouses, which is consistent with workers having more access to meat from pigs and potentially being self infected or exposed to tapeworm carriers.

The HP10 Antigen ELISA is a cost effective tool for screening for cysticercosis as the alternatives are extensive radiological diagnostics (Fleury et al., 2007). Similarly to the taeniasis results, the positive predictive value in this circumstance is likely to be low, reducing confidence in the test results.

4.5 Conclusion

This report is the first to chronicle the prevalence of a range of zoonotic pathogens in slaughterhouse workers in Kenya. A number of these diseases have not been reported in the Kenyan population for many years, with some of the most recent reports of disease from tourists who have been diagnosed in their country of origin (Potasman et al., 2000, Hadda et al., 2009). The results suggest that exposure to these pathogens is occurring in western Kenya which address the first hypothesis of this chapter.

The results indicate that slaughterhouse workers are more exposed to leptospirosis and Q fever than the general population. This result answers the second hypothesis of this chapter. This outcome will be explored in greater detail for these diseases in subsequent chapters. RVF is described in the study area for the first time which raises interesting questions about the maintenance of this pathogen in western Kenya and is a topic that will require further study. The prevalence of brucellosis was much lower than expected and again points to the need for a greater understanding of the maintenance of brucellosis in smallholder farming situations in sub-Saharan Africa.

The prevalence of taeniasis and cysticercosis were lower than expected and disproves the author's hypothesis that slaughterhouse workers may be more exposed to these pathogens through their work.

There is a need for well-performing diagnostic tests in order to make conclusions from serological studies, although at low prevalence even well-performing tests will be affected by low positive predictive values. The majority of tests used for the diagnosis of zoonotic disease are developed and tested in a clinical setting for acute cases where they have a high sensitivity and specificity (Lijmer et al., 1999). In a

clinical setting the persistence of antibodies can be a hindrance to diagnosis, whereas in a serological survey it is valuable to assess the historical exposure to certain pathogens. It is important to note that antibodies reduce with time and titres go down after successful treatment which makes assessment of past exposure difficult in serological surveys (Peter et al., 1988). However, occupational groups that have constant exposure may have higher titres, which is particularly relevant in this circumstance (Cumberland et al., 2001). No conclusions can be made about the current clinical status of workers for this reason.

Many of the diseases tested in this study are misdiagnosed as malaria (Crump et al., 2013, Prabhu et al., 2011). Workers are not likely to know that they have had these zoonotic infections and may receive inappropriate treatment for the conditions (Knobel et al., 2013). Doxycycline is first line treatment for Q fever, brucellosis, and leptospirosis and is a readily available and cheap antibiotic. Tetracyclines (including doxycycline) are the second most dispensed antibiotic in Kenya so it is possible that people are treated effectively without intent or proper diagnosis.

In this communication we have reported seroprevalence of 6 zoonotic diseases in slaughterhouse workers and the limitations to interpretation. Greater awareness regarding the epidemiology of zoonotic diseases in rural settings in sub-Saharan Africa is required in order to develop appropriate control measures. The subsequent chapter will examine risk factors for exposure to zoonotic diseases in slaughterhouse workers in an effort to identify areas to focus control efforts.

Chapter 5

Risk factors for exposure to leptospirosis and Q fever in slaughterhouse workers in western Kenya

5.1 Introduction

Leptospirosis and Q fever in slaughterhouse workers have been identified in prevalence investigation studies and also outbreak response studies in different parts of the world (Campagnolo et al., 2000, Terry et al., 2000, Carrieri et al., 2002, Wilson et al., 2010). The risk factors identified for exposure to leptospirosis in slaughterhouse workers are: smoking and drinking whilst at work, cleaning the intestines, and the role/position of the worker in the slaughterhouse (Chan et al., 1987, Campagnolo et al., 2000, Dreyfus et al., 2014). In contrast, the only risk factor identified for Q fever in slaughterhouse workers is the position or job within the slaughterhouse (Wilson et al., 2010, Riemann et al., 1975).

In this chapter, risk factors for leptospirosis and Q fever exposure in slaughterhouse workers in western Kenya are explored. The hypotheses tested are:

1. work position/role in the slaughterhouse is a risk for zoonotic disease seropositivity in slaughterhouse workers
2. inadequate facilities and poor sanitation in slaughterhouses are risk factors for zoonotic disease seropositivity in workers
3. poor personal hygiene at slaughterhouses are risks for zoonotic disease seropositivity in workers.

The aim of this study is to identify practices that predispose workers to zoonotic disease and to recommend control measures to reduce exposure to these pathogens.

5.2 Methods

5.2.1. Study area and population

The study site and location of slaughterhouses is shown in Figure 3.1. The recruitment of slaughterhouse workers from the participating slaughterhouses is described in Section 2.5.

5.2.2. Data collection

Data collection and sample collection procedures are described in Section 2.5.

5.2.3 Laboratory analysis

Parasitological analyses of blood and faecal samples were conducted at the ILRI laboratory in Busia as described in Section 2.6. At the ILRI laboratory in Nairobi serum samples were tested for leptospirosis and Q fever. The Panbio Leptospira IgM ELISA (Alere, Sinnamon Park, Australia) was used to test serum samples for *Leptospira* antibodies (Winslow et al., 1997). The Serion ELISA Classic *Coxiella burnetii* IgG phase 2 (Virion/Serion. Würzburg, Germany) was used to test for Q fever (Peter et al., 1988). The diagnostic tests are described in detail in Section 2.7.2 and Section 2.7.3. EDTA samples were tested for HIV using the SD Bioline test (Standard Diagnostics Inc) (Vijayakumar et al., 2005).

5.2.4 Statistical analysis

Questionnaire data and laboratory results were entered into Microsoft Access® 2007 databases. Statistical analysis was performed in R (http://CRAN.R-project.org/).

5.2.5 Logistic regression model

Multivariable logistic regression models were used to identify risk factors for leptospirosis and Q fever in slaughterhouse workers and estimate the strength of the relationship with the outcome. The steps in the development of the multivariable model were:

1. Univariable logistic regression was used to compare slaughterhouse types to determine if there was a significant difference between the odds for exposure dependant on the type of slaughterhouse
2. Univariable logistic regression was used to screen variables against disease exposure at the individual level. Variables were included from both the individual and slaughterhouse level data. The variables used are listed in Appendix 8.

   Variables were excluded from analysis if they were strongly correlated with another variable of interest to avoid multicollinearity problems and model estimate instability. Correlation analysis for categorical variables was performed by calculating the phi coefficient of correlation in the *psych* package (Revelle, 2014) in R. Paired variables with a phi coefficient >0.5 were considered highly correlated and the variable that generated the highest *p*-value during univariable logistic regression analysis was excluded.

3. Variables with a *p*-value <0.2 in the univariable analysis were used to develop a multivariable logistic regression model for each exposure. A multivariable mixed effects logistic regression model was used to account

for the clustering of the workers within slaughterhouses. The model was developed using *glmer* function in the *lme4* package (Bates, 2014).

4. A backwards stepwise approach was used for model selection. Starting with a full model using all predictors, variables with the highest *p*-value were dropped in a stepwise fashion. This process was repeated until the model with the lowest Akaikes second-order information criterion (AIC) was identified.
5. The ORs, CIs, and *p*-values were calculated from the model.

5.2.6 Diagnostic checking of multivariable mixed effects model

The traditional approaches for model checking procedures cannot account for the random effects in mixed effects models. Therefore a number of different approaches were used for model checking.

1. Variance Inflation Factors (VIFS) were calculated to check for collinearity. VIFS >4 were considered a problem and the variable removed from the model
2. Moran's I were calculated to check for spatial autocorrelation using the *ape* package (Paradis E., 2004)
3. Histograms of the group level residuals were made to check for normality
4. The mixed effects models were simplified to the basic model that included only the disease outcome and the slaughterhouse. A second model was made using only the individual level factors and finally the complete fitted multivariable mixed effect model. Median odds ratios (MOR) were calculated for the three models and compared (Merlo et al., 2005)

$$MOR = exp\sqrt{2xV_A}x\ 0.6745$$

5. Percentage change in variance (PCV) was calculated between the three models (Merlo et al., 2005)

$$PCV = \frac{V_A - V_B}{V_A}\ x\ 100$$

V_A=variance in the empty model and V_B=variance of model with explanatory variables

5.3 Results

5.3.1 Leptospirosis

a. Comparison between slaughterhouse types

The OR for leptospirosis seropositivity in workers from cattle only slaughterhouses was 0.99 and in workers from pig only slaughterhouses was 1.00 when compared to workers from mixed ruminant slaughterhouses. There was not a significant difference between slaughterhouse types so logistic regression models were created with all slaughterhouse workers as one group (Table 5.1).

Slaughterhouse type	OR (95% CI)	*p*-value
Mixed ruminant	1	Ref
Cattle only	0.99 (0.56–1.75)	0.960
Pig only	1.00 (0.52–1.89)	0.989

Table 5.1 Odds ratios for leptospirosis between slaughterhouse types

b. Univariable logistic regression

The complete univariable analysis for risk factors for leptospirosis seropositivity in slaughterhouse workers included 100 potential exposure variables. Table 5.2 lists the variables regarding personal history that had a *p*-value <0.2 in the univariable analysis. Variables that were significantly associated with leptospirosis seropositivity in slaughterhouse workers after univariable analysis were: having wounds at the time of examination (OR 3.2; 95% CI 1.7–6.0); being HIV positive (OR 0.3; 95% CI 0.1–0.8); smoking (OR 1.7; 95% CI 1.1–2.8) and drinking alcohol (OR 1.7; 95% CI 1.0–2.7).

Variable	% population (n)	% positive (n)	OR (95% CI) n=737	*p*–value
Other job				
Other	59.3 (437)	15.3 (67)	1	Ref
Butcher	40.7 (300)	10.7 (32)	0.6 (0.4–1.0)	0.063
Goat contact outside of work				
No	59.7 (440)	15.0 (66)	1	Ref
Yes	40.3 (297)	11.1 (33)	0.7 (0.4–1.1)	0.133
Pig contact outside of work				
No	63.1 (465)	15.3 (71)	1	Ref
Yes	36.9 (272)	10.3 (28)	0.6 (0.4–1.1)	0.055
Pigs owned				
No	70.0 (516)	14.7 (76)	1	Ref
Yes	30.0 (221)	10.4 (23)	0.67 (0.4–1.1)	0.136
Private borehole for water in the home				
No	91.9 (677)	12.9 (87)	1	Ref
Yes	8.1 (60)	0.2 (12)	1.7 (0.9–3.5)	0.132
HIV				
No	87.9 (648)	14.7 (95)	1	Ref
Yes	12.1 (89)	4.5 (4)	0.3 (0.1–0.8)	**0.013**
Have wounds at the time of examination				
No	92.4 (681)	12.0 (82)	1	Ref
Yes	7.6 (56)	30.4 (17)	3.2 (1.7–6.0)	**<0.001**
Clinic visit in past 3 months				
No	82.5 (608)	14.5 (88)	1	Ref
Yes	17.5 (129)	8.5 (11)	0.6 (0.3–1.1)	0.086
Smoke				
No	76.5 (564)	11.7 (66)	1	Ref
Yes	23.5(173)	19.1 (33)	1.7 (1.1–2.8)	**0.024**
Take alcohol daily				
No	37.3 (275)	9.8 (27)	1	Ref
Yes	62.7 (462)	15.6 (72)	1.7 (1.0–2.7)	**0.040**

Table 5.2 Odds ratios for leptospirosis in slaughterhouse workers examining personal history variables and health

Table 5.3 lists the variables regarding individual slaughterhouse practices that had a *p*-value <0.2 in the univariable analysis screening. Variables that were significantly associated with leptospirosis seropositivity in slaughterhouse workers after univariable analysis were: cleaning the intestines (OR 3.2; 95% CI 1.6–6.7); eating at the slaughterhouse (OR 1.7; 95% CI 1.0–3.0); working at a slaughterhouse where animals were pre-examined before slaughter (OR 0.6; 95% CI 0.4–1.0).

Variable	% population (n)	% positive (n)	OR (95% CI) n=737	*p*–value
Job in the slaughterhouse				
Other	94.3 (695)	12.4 (86)	1	Ref
Cleans intestines	5.7 (42)	31.0 (13)	3.2 (1.6–6.7)	**0.001**
Wear protective clothing				
No	30.8 (227)	16.7 (38)	1	Ref
Yes	69.2 (510)	12.0 (61)	0.6 (0.4–1.0)	0.071
Eat at the slaughterhouse				
No	80.5 (593)	12.1 (72)	1	Ref
Yes	19.5 (144)	18.8 (27)	1.7 (1.0–3.0)	**0.049**
Antemortem exam				
No	55.8 (411)	15.8 (65)	1	Ref
Yes	44.2 (326)	10.4 (34)	0.6 (0.4–1.0)	**0.048**

Table 5.3 Odds ratios for leptospirosis in slaughterhouse workers examining individual slaughterhouse practices

Table 5.4 lists the variables regarding slaughterhouse level practices that had a *p*-value <0.2 in the univariable analysis screening. Variables that were significantly associated with leptospirosis seropositivity in slaughterhouse workers after univariable analysis were: working in a slaughterhouse with more than 5 workers (OR 1.9; 95% CI 1.0–3.8); and working in a slaughterhouse where workers wear protective clothing (OR 0.6; 95% CI 0.3–0.9).

Variable	% population (n)	% positive (n)	OR (95% CI) n=737	*p*–value
Number of workers				
<5	23.9 (176)	8.5 (15)	1	Ref
>5	76.1 (560)	15.0 (84)	1.9 (1.0–3.8)	**0.036**
Number of animals slaughtered				
<8	52.4 (386)	11.1 (386)	1	Ref
≥9	47.6 (350)	16.0 (350)	1.6 (1.0–2.6)	0.071
Roof present				
No	18.2 (134)	8.2 (11)	1	Ref
Yes	81.8 (602)	14.6 (88)	2.0 (1.0–4.2)	0.060
Sides				
No	12.1 (89)	6.7 (6)	1	Ref
Yes	87.8 (647)	12.6 (93)	2.4 (0.9–6.1)	0.066
Water source				
Other	81.8 (603)	12.4 (75)	1	Ref
Well/Spring	18.1 (133)	18.0 (24)	1.7 (0.9–3.1)	0.086
Protective clothing worn				
No	21.7 (160)	19.4 (31)	1	Ref
Yes	78.2 (576)	11.8 (68)	0.6 (0.3–0.9)	**0.028**

Table 5.4 Odds ratios for leptospirosis in slaughterhouse workers examining slaughterhouse factors

The 20 variables from Tables 5.2, 5.3 and 5.4 were identified for inclusion in the multivariable mixed effects logistic regression model. Variables that were obviously correlated to another variable of interest were excluded from the model. Two variables were excluded immediately for being highly correlated to another variable. Having walls in the slaughterhouse was correlated with having a roof (phi coefficient = 0.76). Owning pigs was correlated with having contact with pigs outside the slaughterhouse (phi coefficient = 0.69). The remaining variables were checked for correlation. Figure 5.1 is a correlation matrix for the selected variables. There was a moderate level of correlation between the number of animals slaughtered and the number of people working in the slaughterhouse (phi = 0.42).

	Goat	Pigs	Bore	HIV	Wou	Clinic	Cove	PPE	Butc	Clea	Ante	Smo	Alco	PN	AN	Eat	Roof	Sprin
Goat		0.22	0.05	0.02	0.00	0.02	0.05	0.10	-0.03	0.12	-0.01	0.02	-0.02	0.10	0.07	0.01	0.08	-0.03
Pigs			0.05	0.01	-0.03	0.04	-0.09	-0.14	-0.19	0.04	0.00	-0.05	0.00	-0.12	-0.06	0.05	-0.15	0.09
Bore				0.03	-0.02	-0.02	-0.01	-0.05	0.02	0.04	-0.08	0.00	-0.03	-0.18	-0.02	0.04	-0.13	-0.15
HIV					-0.01	0.11	0.07	0.08	0.00	0.02	-0.02	0.08	0.05	0.00	-0.02	-0.01	0.01	0.01
Wou						0.01	0.07	-0.02	0.04	-0.08	0.00	0.12	0.07	0.07	0.05	0.01	0.03	-0.06
Clinic							0.04	0.01	-0.02	-0.04	0.03	-0.04	-0.03	0.07	-0.01	0.03	0.02	0.03
Cove								0.40	-0.03	-0.01	0.12	0.02	0.02	0.18	0.14	0.00	0.18	-0.03
PPE									0.07	-0.09	0.08	-0.01	-0.01	0.29	0.21	0.01	0.36	0.01
Butc										-0.03	0.08	0.11	0.00	0.02	0.01	0.07	0.11	0.04
Clea											-0.05	0.03	-0.02	-0.08	-0.05	0.00	-0.02	0.02
Ante												-0.03	-0.01	0.09	0.08	0.03	0.12	0.03
Smo													0.25	-0.07	0.00	-0.01	0.04	-0.04
Alco														-0.05	-0.02	0.04	0.03	0.04
PN															**0.42**	-0.04	0.34	-0.02
AN																0.20	0.37	-0.16
Eat																	-0.07	0.10
Roof																		0.13
Sprin																		

* Variable descriptions are included in Table 5.5

Alco = takes alcohol regularly; AN = number of animals in slaughterhouse; Ante = antemortem inspection performed; Bore = worker's private water source; Butc = Butcher; Clea = cleans intestines; Clinic = visited clinic within 3 months; Cove = wear coveralls at work; Eat = eats in the slaughterhouse; Goat = contact with goats outside work; HIV = HIV positive; Pigs = contact with pigs outside work; PN = number of people in slaughterhouse; PPE = works in a slaughterhouse where coveralls worn; Roof= slaughterhouse has a roof; Smo = smokes regularly; Wou = wound at interview; Sprin = Spring is water source

Figure 5.1 Correlation matrix for the selected variables for exposure to leptospirosis in slaughterhouse workers

c. Multivariable logistic regression

Table 5.5 demonstrates the backward stepwise process of model selection from the first model to one step past the model of best fit. The final multivariable model for leptospirosis seropositivity in individual slaughterhouse workers is in bold. This model has an AIC value of 531.45.

Multivariable model	AIC
Leptoresult1 ~ Butc + Goat + Pigs + Bore + AN + Cove + PPE+ Clea + Alco + HIV + Wou + Clinic + Roof + Ante + Smo + PN + Eat + Well + (1 | slaughterhouse_id), family=binomial(logit),data=leptomerge	537.44
Leptoresult1 ~ Butc + Goat + Pigs + Bore +Cove + PPE+ Clea + Alco + HIV + Wou + Clinic + Roof + Ante + Smo + PN + Eat + Well + (1 | slaughterhouse_id), family=binomial(logit),data=leptomerge	535.57
Leptoresult1 ~ Goat + Pigs + Bore + Cove + PPE+ Clea + Alco + HIV + Wou + Clinic + Roof + Ante + Smo + PN + Eat + Well + (1 | slaughterhouse_id), family=binomial(logit),data=leptomerge	534.04
Leptoresult1 ~ Pigs+ Bore + Cove + PPE+ Clea + Alco + HIV + Wou + Clinic + Roof + Ante + Smo + PN + Eat + Well + (1 | slaughterhouse_id), family=binomial(logit),data=leptomerge	532.70
Leptoresult1 ~ Pigs + Cove + PPE + Clea + Alco + HIV + Wou + Clinic + Roof + Ante + Smo + PN + Eat + Well + (1 | slaughterhouse_id), family=binomial(logit),data=leptomerge	531.88
Leptoresult1 ~ Pigs + Cove + PPE + Clea + HIV + Wou + Clinic + Roof + Ante + Smo + PN + Eat + Well + (1 | slaughterhouse_id), family=binomial(logit),data=leptomerge	531.46
Leptoresult1 ~ Pigs + PPE + Clea + HIV + Wou + Clinic + Roof + Ante + Smo + PN + Eat + Well + (1 | slaughterhouse_id), family=binomial(logit),data=leptomerge	**531.45**
Leptoresult1 ~ PPE + Clea + HIV + Wou + Clinic + Roof + Ante + Smo + PN + Eat + Well + (1 | slaughterhouse_id),family=binomial(logit),data=leptomerge	533.11

Alco = takes alcohol regularly; AN = number of animals in slaughterhouse; Ante = antemortem inspection performed; Bore = worker's private water source; Butc = Butcher; Clea = cleans intestines; Clinic = visited clinic within 3 months; Cove = wear coveralls at work; Eat = eats in the slaughterhouse; Goat = contact with goats outside work; HIV = HIV positive; Pigs = contact with pigs outside work; PN = number of people in slaughterhouse; PPE = works in a slaughterhouse where coveralls worn; Roof= slaughterhouse has a roof; Smo = smokes regularly; Wou = wound at interview

Table 5.5 Multivariable model selection for leptospirosis in slaughterhouse workers.

Variable	OR (95% CI)	*p* value	VIFs
Individual factors			
Cleans intestines	3.8 (1.8–8.2)	<0.001	1.055
Wounds	2.7 (1.4–5.3)	0.004	1.041
Smoking	1.8 (1.1–3.0)	0.016	1.042
Eating	2.1 (1.2–3.6)	0.006	1.051
HIV positive	0.3 (0.1–0.9)	0.036	1.013
Visited a clinic in past 3 months	0.5 (0.2–1.0)	0.045	1.036
Worker reports antemortem inspection performed	0.6 (0.4–0.9)	0.028	1.037
Contact with pigs outside work	0.6 (0.4–1.0)	0.061	1.054
Slaughterhouse level factors			
Slaughterhouse has a roof	2.6 (1.2–5.7)	0.016	1.241
Greater than 5 workers	2.4 (1.2–4.7)	0.012	1.193
Well or spring as water source for the slaughterhouse	2.1 (1.2–3.6)	0.010	1.077
Workers wear protective clothing in slaughterhouse	0.3 (0.2–0.5)	<0.001	1.400

Table 5.6 Results of multivariable analysis for leptospirosis in slaughterhouse workers

The results of the multivariable logistic regression for leptospirosis seropositivity in slaughterhouse workers are shown in Table 5.6. Risk factors on an individual level that were significant for exposure to leptospirosis were: cleaning intestines (OR 3.8; 95% 1.8–8.2); having a wound at interview (OR 2.7; 95% CI 1.4–5.3); smoking (OR 1.8; 95% CI 1.1–3.0); eating at the slaughterhouse (OR 2.1; 95% CI 1.2–3.6). Individual factors that were protective against exposure were: being HIV positive (OR 0.3; 95% CI 0.1–0.9); performing antemortem inspection of animals (OR 0.6; 95% CI 0.4–0.9); seeking health care by visiting a clinic (OR 0.5; CI 95% 0.2–1.0). At the slaughterhouses level, factors that were significant for individual exposure risk include: working in a slaughterhouse with a roof (OR 2.6; 95% CI 1.2–5.7);

working in slaughterhouse with more than 5 workers (OR 2.4; 95% CI 1.2–4.7) and slaughterhouses that source water from a well or spring (OR 2.1; 95% CI1.2–3.6). Protective factors include working at a slaughterhouse where protective clothing is worn by workers (OR 0.3; 95% CI 0.2–0.5).

d. Model checking

A number of tools were used to check the measure of fit of the model.

The Moran's I calculation demonstrated no evidence of spatial autocorrelation.

The histogram of the group level residuals have a normal distribution (Figure 5.2).

The median OR for the fitted model was equal to 1 demonstrating that there was no variation between slaughterhouses (Table 5.7)

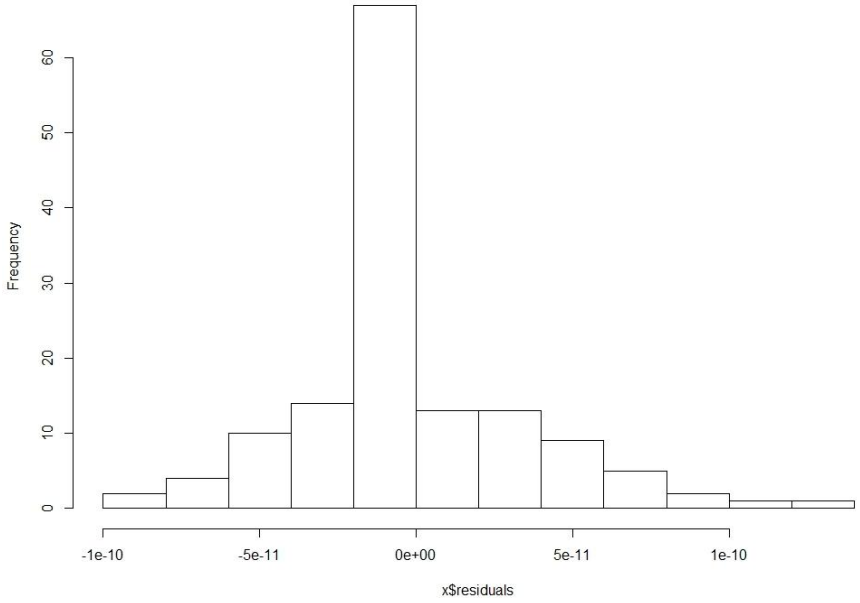


Figure 5.2 Histogram of the group level residuals from model for leptospirosis in slaughterhouse workers

Variable	Empty model	Model with individual variables	Model with individual and SH variable
Individual factors			
Cleans intestines		3.2 (1.5–6.8)**	3.8 (1.8–8.2)***
Wounds		2.8 (1.4–5.4)**	2.7 (1.4–5.3)**
Smoking		1.8 (1.0–2.9)*	1.8 (1.1–3.0)*
Eating		1.8 (1.0–3.1)*	2.1 (1.2–3.6)**
HIV positive		0.3 (0.1–0.8)*	0.3 (0.1–0.9)*
Visited a clinic in past 3 months		0.6 (0.3–1.1)	0.5 (0.2–1.0)*
Worker reports antemortem inspection performed		0.6 (0.4–1.0)*	0.6 (0.4–0.9)*
Contact with pigs outside work		0.6 (0.4–1.0)	0.6 (0.4–1.0)
Slaughterhouse level factors			
Slaughterhouse has a roof			2.6 (1.2–5.7)*
Greater than 5 workers			2.4 (1.2–4.7)*
Well or spring as water source			2.1 (1.2–3.6)*
Workers wear protective clothing in slaughterhouse			0.3 (0.2–0.5)***
Variance (SE)	0.33 (0.02)	0.20 (0.02)	2.5×10^{-11}
Proportional change in variance	Reference	39.4%	99.9%
Median OR	1.7	1.5	1.0
AIC	582.8	552.0	531.4

*****p <0.05, ******p < 0.01, and *******p < 0.001

Table 5.7 Individual and slaughterhouse level predictors associated with leptospirosis in slaughterhouse workers by multivariable logistic regression

5.3.2 Q fever

a. Comparison between slaughterhouse types

The OR for Q fever seropositivity in workers from cattle only slaughterhouses was 0.65 and in workers from pig only slaughterhouses was 0.36 when compared to workers from mixed ruminant slaughterhouses. There was not a significant difference between slaughterhouse types (Table 5.8). A model was developed that analysed ruminant and cattle slaughterhouses without the pig slaughterhouses since being a pig slaughterhouse worker is not a risk factor for exposure to Q fever and any exposures in this group were likely to happen outside the slaughterhouse (Australian Government, 2013).

Variable	OR (95% CI)	*p*-value
Mixed ruminant	1	Ref
Cattle only	0.65 (0.30–1.39)	0.266
Pig only	0.36 (0.12–1.09)	0.072

Table 5.8 Odds ratios for Q fever amongst slaughterhouse workers between slaughterhouse types

b. Univariable logistic regression

The complete univariable analysis for risk factors for Q fever seropositivity in slaughterhouse workers included 100 potential exposure variables. Table 5.9 lists the variables regarding personal history that had a *p*-value <0.2 in the univariable analysis. The only significant variable from the univariable analysis associated with Q fever seropositivity in slaughterhouse workers was using municipal water for personal consumption (OR 2.9; 95% CI 1.2–-7.1).

Variable	% population (n)	% positive (n)	OR (95%CI) n=737	*p*–value
Take alcohol weekly				
No	37.3 (275)	2.5 (7)	1	Ref
Yes	62.7 (462)	5.6 (26)	2.28 (0.98–5.33)	0.057
Intoxicated at interview				
No	88.0 (497)	4.6 (23)	1	Ref
Yes	12.0 (68)	8.8 (6)	2.0 (0.8–5.1)	0.151
Sheep owned				
No	72.9 (412)	4.4 (18)	1	Ref
Yes	27.1 (153)	7.2 (11)	1.7 (0.8–3.7)	0.181
Municipal water for personal use				
No	87.1 (492)	4.3 (21)	1	Ref
Yes	12.9 (73)	11.0 (8)	2.9 (1.2–7.1)	**0.021**
HIV				
No	86.4 (488)	4.5 (22)	1	Ref
Yes	13.6 (77)	9.1 (7)	2.1 (0.9–5.3)	0.10
Clinic visit				
No	82.3 (465)	5.8 (27)	1	Ref
Yes	17.7 (100)	2.0 (2)	0.3 (0.1–1.4)	0.139

Table 5.9 Odds ratios for Q fever in slaughterhouse workers examining personal history variables and examining health factors

Table 5.10 lists the variables regarding individual slaughterhouse practices that had a *p*-value <0.2 in the univariable analysis. Table 5.11 lists the variables regarding slaughterhouse level factors that had a *p*-value <0.2 in the univariable analysis.

Variable	% population (n)	% positive (n)	OR (95% CI) n=737	*p*–value
Coveralls				
No	23.0 (130)	7.7 (10)	1	Ref
Yes	77.0 (435)	4.4 (19)	0.54 (0.2–1.2)	0.137
Boots				
No	46.0 (260)	6.9 (18)	1	Ref
Yes	54.0 (305)	3.6 (11)	0.5 (0.2–1.1)	0.080
Wash hands before slaughter				
No	31.5 (178)	7.3 (13)	1	Ref
Yes	68.5 (387)	4.1 (16)	0.5 (0.2–1.2)	0.121
Wash hands after slaughter				
No	5.7 (32)	13.0 (4)	1	Ref
Yes	94.3 (533)	4.7 (25)	0.3 (0.1–1.1)	0.063
Wash hands after latrine				
No	41.6 (235)	6.8 (16)	1	Ref
Yes	58.4 (330)	3.9 (13)	0.6 (0.3–1.2)	0.134

Table 5.10 Odds rations for Q fever in slaughterhouse workers examining individual slaughterhouse practices

Variable	% population (n)	% positive (n)	OR (95% CI) n=737	*p*–value
Number of animals				
<8	49.9 (282)	6.4 (18)	1	Ref
≥8	50.1 (283)	3.9 (11)	0.6 (0.3–1.3)	0.183
Total people				
<10	49.7 (281)	6.8 (19)	1	Ref
≥10	50.3 (284)	3.5 (10)	0.5 (0.2–1.1)	0.086
PPE				
No	11.5 (65)	9.2 (6)	1	Ref
Yes	88.5 (500)	4.6 (23)	0.5 (0.2–1.2)	0.119
Boots				
No	18.1 (102)	7.8 (8)	1	Ref
Yes	81.9 (463)	4.5 (21)	0.6 (0.2–1.3)	0.176

Table 5.11 Odds ratios for Q fever in slaughterhouse workers examining slaughterhouse factors

The 15 variables from Tables 5.9, 5.10, and 5.11 were identified for inclusion in the multivariable mixed effects logistic regression model. Figure 5.3 is a correlation matrix for the selected variables. There was a high level of correlation between the number of animals slaughtered and the number of people working in the slaughterhouse (phi = 0.68). The number of animals slaughtered was excluded from the model. There was a high correlation between slaughterhouses where workers wore protective clothes and slaughterhouses where workers wore rubber boots. Rubber boots at the slaughterhouse level was excluded from the model (phi = 0.58).

	AN	PN	Sheep	Alcoho	Munic	Clinic	Cover	Boots	HIV	Before	After	AfterL	Drunk	PPE	SHB
AN		**0.68**	0.07	-0.04	0.09	-0.01	0.19	0.36	-0.03	0.11	0.04	0.11	-0.01	0.32	0.35
PN			0.05	0.01	0.10	-0.05	0.21	0.32	-0.04	0.04	0.12	0.09	0.03	0.31	0.32
Sheep				-0.02	-0.03	0.01	0.03	0.07	-0.01	0.09	0.05	0.11	-0.08	0.03	0.02
Alcoho					-0.07	-0.04	0.01	0.04	0.08	-0.07	-0.02	-0.1	0.27	-0.01	-0.02
Munic						0.06	0.04	0.02	0.08	0.03	-0.04	0.05	-0.08	0.12	0.04
Clinic							0.03	-0.04	0.09	-0.01	0.05	0.08	0.06	-0.01	-0.05
Cover								0.40	0.06	0.08	0.1	0.19	0.05	0.33	0.28
Boots									0.08	0.08	0	0.09	-0.06	0.31	0.43
HIV										0.06	0.08	0.01	-0.07	0.03	-0.01
Before											0.16	0.12	-0.11	-0.01	0.09
After												0.01	0	-0.06	-0.08
AfterL													0.06	0.04	0.02
Drunk														0	0.03
PPE															**0.58**
SHB															

* Variable descriptions are included in Table 5.12

Figure 5.3 Correlation matrix for variables associated with exposure to Q fever in slaughterhouse workers

c. Multivariable logistic regression

Table 5.12 demonstrates the backward stepwise process of model selection from the first model to one step past the model of best fit. The final multivariable model for Q fever seropositivity in individual slaughterhouse workers is in bold. This model has an AIC value of 222.3.

Multivariable model	AIC
Qresult ~ PN + Sheep + Alco + Munic + Clinic + Cover + Boots + AfterL + After + HIV + Before + PPE + Drunk + (1 | slaughterhouse_id) ,family=binomial(logit), data=leptomerge	227.7
Qresult ~ PN+ Sheep + Alco + Munic + Clinic + Boots + AfterL + After + HIV + Before + PPE + Drunk + (1 | slaughterhouse_id) ,family=binomial(logit), data=leptomerge	225.8
Qresult ~ PN+ Sheep + Alco + Munic + Clinic + AfterL + After + HIV + Before + PPE + Drunk + (1 | slaughterhouse_id) ,family=binomial(logit), data=leptomerge	224.1
Qresult ~ PN+ Sheep + Munic + Clinic + AfterL + After + HIV + Before + PPE + Drunk + (1 | slaughterhouse_id) ,family=binomial(logit), data=leptomerge	222.7
Qresult ~ PN + Sheep + Munic + Clinic + AfterL + After+ HIV + PPE + Drunk + (1 | slaughterhouse_id) ,family=binomial(logit), data=leptomerge	222.3
Qresult ~ Sheep + Munic + Clinic + AfterL + After + HIV + PPE + Drunk + (1 | slaughterhouse_id) ,family=binomial(logit), data=leptomerge)	**222.3**
Qresult ~ Sheep + Munic + Clinic + After + HIV + PPE+ Drunk + (1 | slaughterhouse_id) ,family=binomial(logit), data=leptomerge	223.2

The codes applied are as follows: Alco = takes alcohol regularly; After = washes hand after slaughtering; AfterL = washes hands after latrine; Before = washes hands before slaughtering; Boots = wears boots at works; Clinic = visited clinic in past 3 months; Cover = wears coveralls at work; Drunk = Intoxicated at interview: HIV = HIV positive; Munic = worker's private water source is municipal water; PN = number of people in slaughterhouse; PPE = works in a slaughterhouse where coveralls are worn; SHB = works in a slaughterhouse where boots are worn; Sheep = contact with sheep away from work

Table 5.12 Multivariable model selection for Q fever seropositivity in slaughterhouse workers

The results of the multivariable logistic regression for Q fever seropositivity in slaughterhouse workers are shown in Table 5.13. Risk factors on an individual level for exposure to Q fever were: using municipal water for personal use (OR 4.2; 95% CI 1.5–11.5); being intoxicated at work (OR 3.2; 95% CI 1.1–9.4). Working in a slaughterhouse where workers wear protective clothing is protective against Q fever (OR 0.3; 95% CI 0.1–0.9).

Variables	OR (95% CI)	*p*–value	VIFS
Individual variables			
Sheep contact	2.2 (0.9–5.2)	0.078	1.030
Municipal water for personal use	4.2 (1.5–11.5)	0.005	1.137
Visited clinic in past 3 months	0.3 (0.1–1.2)	0.090	1.026
Wash hands after slaughter	0.3 (0.1–1.0)	0.098	1.045
Wash hands after latrine	0.5 (0.2–1.1)	0.058	1.022
HIV	2.5 (0.9–7.0)	0.072	1.043
Intoxicated at interview	3.2 (1.1–9.4)	0.037	1.058
Slaughterhouse variables			
Workers wear protective clothing at slaughterhouse	0.3 (0.1–0.9)	0.035	1.092

Table 5.13 Results of the multivariable analysis for Q fever in slaughterhouse workers

d. Model checking

A number of tools were used to check the measure of fit of the model.

The Moran's I calculation demonstrated no evidence of spatial autocorrelation.

The histogram of the group level residuals has a normal distribution (Figure 5.4).

The median OR for the fitted model was 1.67 suggesting that if workers moved to a slaughterhouse with a higher risk of Q fever then they have an increased odds of 1.67 of being exposed to Q fever (Table 5.14)

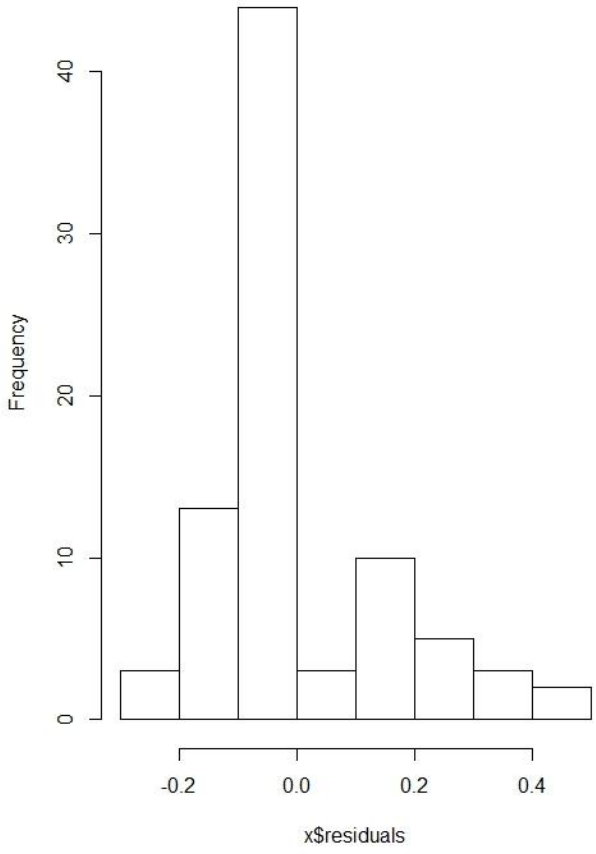


Figure 5.4 Histogram of the group level residuals from the model for Q fever in slaughterhouse workers

Variable	Empty model	Model with individual variables	Model with individual and slaughterhouse variables
Individual variables			
Sheep contact		2.2 (0.9–5.2)	2.2 (0.9–5.2)
Municipal water for personal use		3.5 (1.3–9.5)*	4.2 (1.5–11.5)**
Visited clinic in past 3 months		0.3 (0.1–1.3)	0.3 (0.1–1.2)
Wash hands after slaughter		0.3 (0.1–1.2)	0.3 (0.1–1.0)
Wash hands after latrine		0.5 (0.2–1.2)	0.5 (0.2–1.1)
HIV		2.5 (0.9–6.9)	2.5 (0.9–7.0)
Intoxicated at interview		3.2 (1.1–9.4)*	3.2 (1.1–9.4)*
Slaughterhouse variables			
Workers wear protective clothing at slaughterhouse			0.3 (0.1–0.9)*
Variance (SE)	$1.1x10^{-12}$	0.409	0.287
Proportional change in variance	Reference	100%	100%
Median OR	1.00	1.84	1.67
AIC	232.7	224.3	222.3

*p <0.05, **p < 0.01, and ***p < 0.001

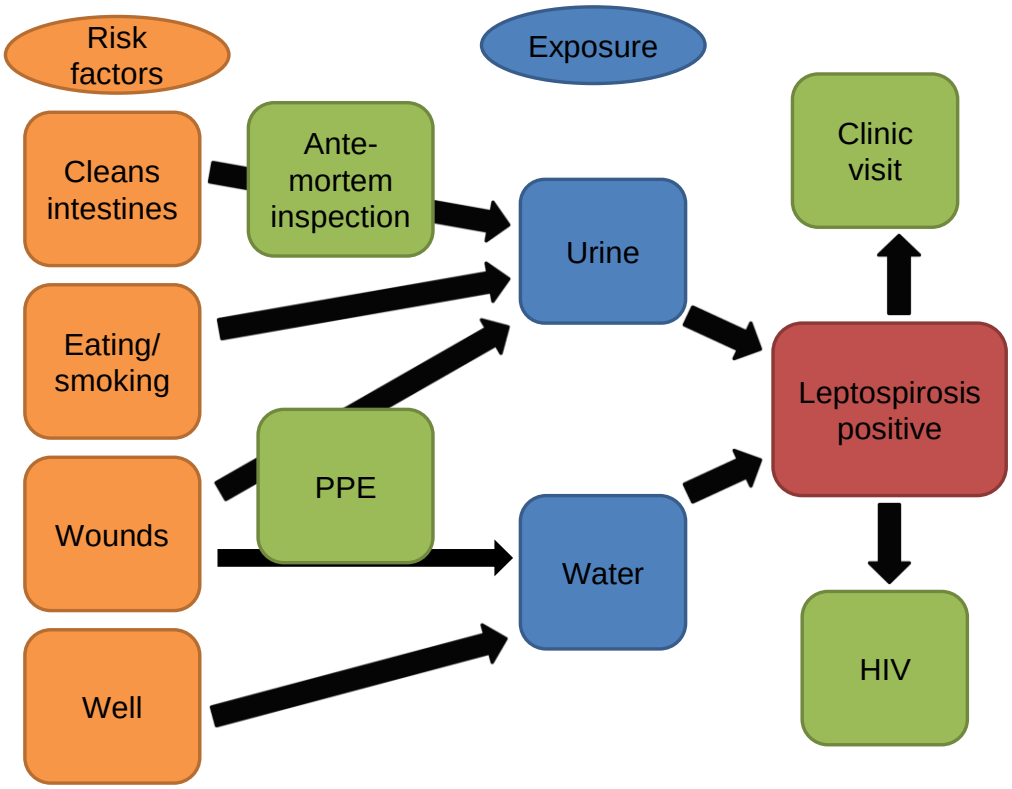
Table 5.14 Individual and slaughterhouse level predictors associated with Q fever in slaughterhouse workers by multivariable logistic regression

5.4 Discussion

5.4.1 Leptospirosis

There was not a statistically significant difference in the risk of leptospirosis seropositivity between workers from different slaughterhouse types, suggesting that the risk for exposure was the same across the three types of slaughterhouse (Table 5.1). This is in contrast to other studies that have shown an increased risk in specific slaughterhouse types, for example, sheep slaughterhouses (Dreyfus et al., 2014).

The multivariable logistic regression analysis demonstrated a number of variables to be associated with leptospirosis seropositivity in slaughterhouse workers (Figure 5.5).



Risk factors (orange), protective factors (green), source (blue), outcome (red)

Figure 5.5 Causal framework of risk factors for leptospirosis in slaughterhouse workers

a. Individual factors

Workers that cleaned the intestines were at increased risk of exposure to leptospirosis compared to workers in other positions in the slaughterhouse (OR 3.8; 95% CI 1.8–8.2). It has been reported that different roles or positions in the slaughterhouse have differing levels of risk for leptospirosis, with those that have contact with the viscera being at higher risk (Dreyfus et al., 2014, Chan et al., 1987).

Eating at the slaughterhouse (OR 2.1; 95% CI 1.2–3.6) and smoking (OR 1.8; 95% CI 1.1–3.0) were shown to be risk factors for exposure to leptospirosis. Similar findings have been reported in pig slaughterhouse workers in the USA where smoking and drinking beverages at work were reported as risk factors for leptospirosis (Campagnolo et al., 2000). The same study in the USA reported that washing hands after work was protective, which was not found in this study.

Workers with wounds were more likely to be seropositive to leptospirosis (OR 2.7; 95% CI 1.4–5.3). This result is consistent with regular pathways of infection through cuts and abrasions (Monahan et al., 2009). Workers in slaughterhouses where protective clothing was worn were at less risk of testing seropositive for leptospirosis (OR 0.3; 95% CI 0.2–0.5). Wearing protective clothing has been shown to be protective for other zoonotic pathogens such as *Brucella* sp (Nabukenya et al., 2013). Since leptospirosis is transmitted through cuts and mucous membrane contact, only protective equipment that covered the hands and face would be protective. Therefore clothing would not necessarily prevent exposure. It is possible the clothing is confounded by another unidentified factor such as greater care or risk aversion as has been seen in other studies (Kwan et al., 2002). This might also explain the reason why workers who visited a clinic in the last 3 months were less likely to test

seropositive to leptospirosis (OR 0.5; 95% CI 0.2–1.0). Workers that proactively seek health care may engage in safer work practices and be more likely to seek treatment for illness.

People with HIV were at reduced risk of exposure to leptospirosis (OR 0.3; 95% CI 0.10.9). This result is similar to that of a hospital based study in Tanzania (Biggs et al., 2013). Biggs et al (2013) did not offer an explanation for this finding and concluded that further investigation of coinfection in HIV and leptospirosis endemic areas was warranted. A study in India showed that mortality was high in coinfected individuals (Kuppalli, 2011). It is possible that high morbidity and mortality in coinfected individuals explains their absence from this study group. An alternative hypothesis is that HIV positive individuals on antiretroviral therapy may have improved access to treatment for opportunistic infections and hence be more likely to receive antibiotics if required.

Workers that reported seeing animals pre-examined before slaughter had a reduced risk of leptospirosis exposure (OR 0.6; 95% CI 0.4–0.9). Animals with leptospirosis can present with fever, inappetence, mastitis, jaundice, anaemia, pneumonia, or abortion. However the vast majority will be asymptomatic and animals can shed *Leptospira* spp. in their urine for a long time after infection (Herenda, 1994). These animals are unlikely to be removed from slaughter due to clinical illness. This finding might be confounded by another unidentified factor.

b. Slaughterhouse factors

Workers that worked in slaughterhouses that have a roof had a higher risk of leptospirosis exposure (OR 2.6; 95% CI 1.2–5.7). Leptospirosis has been shown to

survive in the environment in diluted urine in direct sunlight for 2 days and in cooler shaded environments for longer (Khairani-Bejo, 2004). These findings could suggest that leptospires survive longer in slaughterhouses that have a roof if they are not adequately cleaned, leading to exposure of workers.

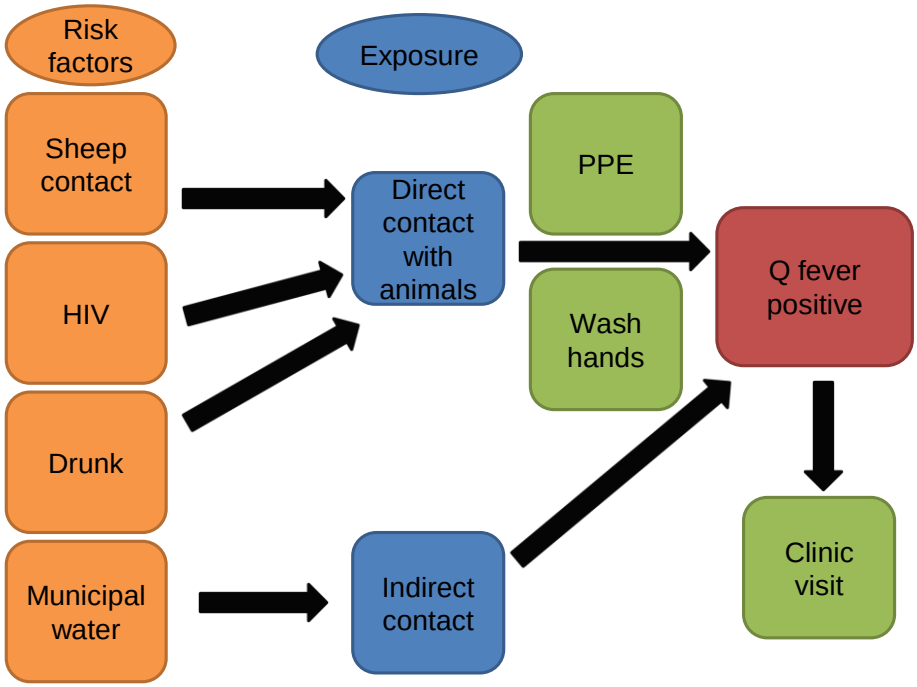
The number of people that worked in a slaughterhouse was also associated with an increased risk of leptospirosis exposure (OR 2.4; 95% CI 1.2–4.7). Larger slaughterhouses will have a higher through-put of animals, increasing the likelihood of infected animals being slaughtered. However, larger slaughterhouses also have better management and hygiene practices as outlined in Chapter 3. It is likely that there is a more complex epidemiology in the larger slaughterhouses that is related to the number and type of animals being slaughtered. Information on the number of infected animals entering the slaughterhouse would be important to quantify the risk to workers.

Using well water at the slaughterhouse was associated with increased exposure to leptospirosis in slaughterhouse workers (OR 2.1; 95% CI 1.2–3.6). Contaminated water can be a source of infection (Levett, 2001).

5.4.2 Q fever

There was not a statistically significant difference in the risk of Q fever seropositivity between workers from different slaughterhouse types. It was considered that pig only slaughterhouse workers would not be exposed to Q fever within the slaughterhouse environment (Table 5.8). Pigs are not considered a reservoir of this pathogen (Stoker and Marmion, 1955). For this reason pig only slaughterhouse workers were excluded from the model.

The multivariable logistic regression analysis demonstrated 3 variables to be significantly associated with Q fever seropositivity in slaughterhouse workers (Figure 5.6). These were using municipal water for personal use, being intoxicated at interview and working in a slaughterhouse where workers wore protective clothing.



Risk factors (orange), protective factors (green), source (blue), outcome (red)

Figure 5.6 Causal framework of risk factors for Q fever in slaughterhouse workers

The multivariable analysis identified workers that used municipal water for personal use to be at increased risk of exposure to Q fever (OR 4.2; 95% CI 1.5–11.5). Q fever is not transmitted by ingestion of water and studies have shown there is a negligible risk of being infected with Q fever by aerosolisation of water (Sales-Ortells and Medema, 2012). Municipal water users must live in larger towns to have access to this water source. Increased Q fever incidence has been associated with urbanisation (Hellenbrand et al., 2001). These Q fever cases associated with metropolitan water use were notably along one of the major road routes to Uganda. This finding needs to be investigated further.

Slaughterhouse workers that appeared intoxicated at interview were at greater risk of Q fever exposure (OR 3.2; 95% CI 1.1–9.4). It was common for workers to consume a locally made fermented porridge, called “nyuka”, “busela”, or “ebusera” before or during work. Alcohol consumption can impair performance and motor skills resulting in injury. Studies in farm workers showed that regular alcohol consumption was associated with increased injury frequency (Stallones and Xiang, 2003). Regular alcohol consumption may also reduce immunity, increasing susceptibility to infection, particularly bacterial pneumonia (Nelson and Kolls, 2002).

Individuals that were HIV positive had 2.5 times the odds of being seropositive to Q fever. People infected with HIV are at greater risk of Q fever co-infection due to immunosuppression (Raoult et al., 1993). Although HIV infection was not a significant variable in the multivariable model it must still be considered an important risk factor for Q fever exposure in slaughterhouse workers in western Kenya.

Workers that worked in a slaughterhouse where protective clothing was worn were less exposed to Q fever (OR 0.3; 95% CI 0.1–0.9). As Q fever is transmitted by aerosols it is unlikely that the protective clothing prevents infection. Similar to the findings for leptospirosis, this result may be confounded by another unidentified factor, such as increased risk aversion.

Contact with infected sheep is considered a risk factor for Q fever (O'Connor et al., 2014). Workers that had contact with sheep had 2.2 times the odds of being seropositive for Q fever. Although this was not a significant variable in the multivariable model contact with sheep should be considered a risk factor for Q fever.

It is interesting to note that the median OR suggests that the there is some effect of the slaughterhouse level clustering on seropositivity for Q fever (OR 1.6) in slaughterhouse workers. However this effect is not as strong as the individual OR for the other variables (municipal water, intoxication, protective clothing).

5.5 Conclusion

This study is the first of its type in Kenya to investigate the risk factors for zoonotic disease exposure in slaughterhouse workers. The study hypothesised that:

1. work position/role in the slaughterhouse was a risk for zoonotic disease seropositivity in slaughterhouse workers
2. inadequate facilities and poor sanitation in slaughterhouses are risk factors for zoonotic disease seropositivity in workers
3. poor personal hygiene at slaughterhouses are risks for zoonotic disease seropositivity in workers.

The findings of this study support these hypotheses. The workers with the greatest risk of leptospirosis seropositivity are those that have contact with the viscera through cleaning the intestines. This seropositivity is likely due to their intimate contact with infected organs.

At the slaughterhouse level, increased leptospirosis risk in workers is associated with infrastructural factors (having a roof) and the source of water (well or spring). These factors are likely to be indicative of poor sanitation at the slaughterhouse. This assumption is further supported by the finding that slaughterhouse workers in slaughterhouses where protective clothing is worn have reduced risk for both leptospirosis and Q fever. In addition, workers that report animals being preexamined before slaughter have reduced leptospirosis risk, further supporting the hypothesis that workers in slaughterhouses with better facilities and practices have reduced zoonotic disease risk.

Personal hygiene factors appear to have the most influence on zoonotic disease risk. Workers that have wounds, smoke, and eat at the slaughterhouse have higher risk for leptospirosis than other workers. In addition, workers that are intoxicated at work have higher Q fever risk than other workers.

In order to improve conditions in slaughterhouses in western Kenya and reduce exposure of workers to zoonotic diseases, improvements need to be made to slaughterhouse facilities. Workers need to be educated regarding their disease risks and ways to prevent or reduce transmission. Areas that need to be targeted for intervention include:

1. Sanitation
    a. Regular cleaning of the slaughterhouse with disinfectant
    b. Potable water source
    c. Hand washing facilities
    d. Provision of personal protective equipment to workers
2. Personal hygiene
    a. Prevent eating and smoking at work
    b. Workers to wear protective clothing
    c. Wounds to be covered
3. Meat inspection
    a. Antemortem inspection
4. Slaughterhouse worker health
    a. Regular medical check ups

A detailed report of the findings of this study will be prepared for the local veterinary department and the workers regarding recommended measures to reduce zoonotic disease risk in slaughterhouse workers in western Kenya.

Chapter 6

Epidemiology of leptospirosis and Q fever in people in western Kenya

6.1 Introduction

Risk factors for zoonotic disease exposure are commonly direct contact with animals but there can be indirect exposures, particularly for waterborne diseases such as leptospirosis, or aerosols such as Q fever (Waitkins, 1986, Campagnolo et al., 2000, Raoult and Marrie, 1995, Marmion, 1959).

6.1.1 Leptospirosis

People who come into contact with animal urine, such as farmers, and those that have contact with contaminated water, such as sewer workers are at risk of leptospirosis (Waitkins, 1986). In Kenya, rodents, cattle, goats, and sheep have been shown to be maintenance hosts of leptospires (Halliday et al., 2013, Ball, 1966).

6.1.2. Q fever

People who are in contact with peri-parturient animals such as farmers, veterinarians, and slaughterhouse workers are considered to be most at risk, but people who live near farms can also be affected through dispersal of the pathogen (Stoker and Marmion, 1955, Roest et al., 2011). In Kenya, Q fever has been described in cattle, sheep, goats and camels (Depuy et al., 2014).

In this chapter, risk factors for leptospirosis and Q fever exposure in the general community are explored. The hypothesis tested is:

1. People in contact with animals have greater risk of zoonotic disease.

The aim of this study was to identify risk factors for zoonotic disease exposure in the community and compare these to the risk factors identified for zoonotic disease exposure in slaughterhouse workers.

6.2 Methods

6.2.1 Sampling frame

The data used for this paper incorporates information from the People, Animals and their Zoonoses (PAZ) study (Doble and Fevre, 2010). PAZ was a cross-sectional study of zoonoses in people and animals in western Kenya. The study area was the same as described in Section 2.1. The population of the study area was 1.4 million people living in 240,004 homesteads (estimated from the Kenyan Human Population Census of 2009). The estimated livestock population of 557,418 cattle and 68,484 pigs was obtained from the Divisional Livestock Production Office (DLPO).

The sample size was calculated for an expected prevalence of 2% *C. burnetii* in cattle using the equation (Dohoo, 2003):

$$n = deff\frac{z^2pq}{e^2}$$

n= sample size
deff = design effect (5)
z =the confidence interval for a normal distribution taken as 1.96 (95% level)
p =the proportion disease expected in the population
q=1-p
e=the level of precision (5%)

The sample size was calculated as 2300 cattle to be sampled from 412 homesteads given an average herd size of 5 animals. The study was designed to compare the seroprevalence of zoonotic diseases in livestock keeping houses to that in non livestock keeping households. In order to recruit livestock keeping households the study area was stratified according to the cattle population density which was calculated from the 2005 livestock census and inflated by 10% per year (Figure 6.1).

The selection of homesteads was done using a two stage cluster design. The study area was divided into 164 sublocations, which is the smallest administrative unit in Kenya. The number of homesteads selected from each sublocation was proportional to the cattle density i.e. more households were sampled in sub-locations with more livestock. The human sample size was the number of individuals living in the selected homesteads.

A random set of points was generated within each sublocation using ArcMap™ version 9.1 and the extension Hawths tools. Maps were provided by the ILRI geographical information systems unit (http://www.ilri.org/gis/). A handheld GPS Garmin eTrex®, was used in the field to locate each point. The nearest homestead within 300 metres of the point was recruited into the study. If there were no homesteads in the area or the homestead head refused to participate then a backup point was used. The homestead head was advised of the study aims and objectives and recruited into the study, and an appointment made for data collection and sampling the following week.

6.2.2 Sample collection

On the day of sampling, all homesteads members aged over 5 years were invited to participate. Exclusion criteria included severe anaemia and third trimester pregnancy. Each participant was individually interviewed with a structured questionnaire that included questions regarding demographic data, health, and risk factors for zoonotic disease exposure (Appendix 9). Participants were given a clinical examination by a trained clinical officer assigned to the project and data were recorded regarding height, weight, temperature, anaemia, jaundice, and organ enlargement. Participants

under the age of 14 were supervised by an adult relative. The homestead head was asked a homestead-level questionnaire regarding animal ownership, wealth indicators, water source, and access to healthcare (Appendix 10). Blood and faecal samples were collected as described for slaughterhouse workers in Section 2.5.

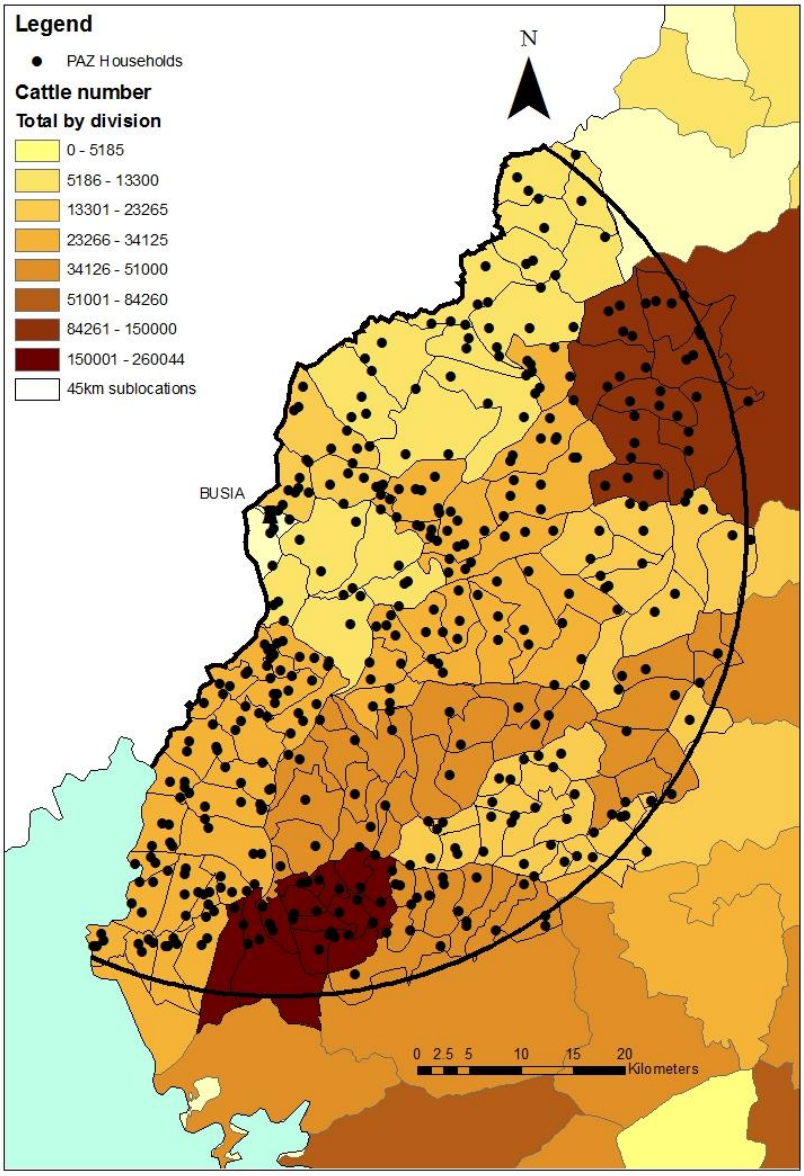


Figure 6.1 Map of the study area indicating the cattle population numbers for each division, the sublocation boundaries and the selected homesteads

6.2.3 Sample analysis

Human samples were prepared and tested in the Busia laboratory for blood and faecal parasites as described in Section 2.6. In addition, faecal samples were examined for evidence of parasitism using the Kato–Katz technique (Cheesbrough, 2006). Samples were transported to the ILRI Nairobi laboratory for further serological testing for HIV, leptospirosis, and Q fever as described in Section 2.7.

6.2.4 Data management

Data were recorded in a Palm operating system (Palm OS) personal digital assistant (PDA) using Pendragon Forms 5.1 (Pendragon Software Corporation). Microsoft® Access databases were used to manage data.

Variables were recoded in R software version 3.0.2 (R Core Team, 2013). Variables that were recoded for the purposes of analysis:

- Age was coded as a binary variable less or equal to the median age of 35 years.
- Variables relating to frequency of an event were recoded as binary variables to Always/Sometimes versus Rarely/Never.

6.2.5 Spatial analysis

For mapping purposes homesteads were considered positive for each pathogen if one or more inhabitants were positive for leptospirosis or Q fever, respectively. The locations of homesteads were mapped using ArcGIS^{TM} (ESRI, Redlands, CA, USA).

6.2.6 Data analysis

For the purposes of this comparison, the population aged over 20 were selected from the PAZ cross-sectional study (referred to from here as the PAZ adjusted

population). This population was selected for its resemblance to the slaughterhouse worker population. This process was done in order to reduce any selection bias or "healthy worker effect" when comparing this population to the slaughterhouse workers population (Shah, 2009). A subset of questions from the individual questionnaire regarding demographics, health, and risk factors for zoonotic disease exposure were used for analysis.

The *Survey* package (Lumley, 2012, Lumley, 2004) in R was used to adjust for clustering. Sampling weights were calculated by dividing the number of people per division (from the Kenyan Human Population Census of 2009) by the number of people sampled in each division. The *svydesign* function was used to calculate a design effect using the following equation. The design effect was used to calculate adjusted proportions for survey responses.

$$designobject <- svydesign(id = \sim homestead\_id, weights = \sim weights, data = pazdata, fpc = \sim fpc)$$

The apparent seroprevalence estimates for leptospirosis and Q fever were calculated using the *epi.prev* function in the *EpiR* package (Stevenson, 2014b) of R (http://CRAN.R-project.org/). The seroprevalence results were adjusted using homestead as a clustering variable in the *Survey* package of R. The above design object was used to calculate an adjusted prevalence estimate accounting for the clustering of the data. The true prevalence estimate accounting for the test sensitivity and specificity was calculated using *truePrev* function in the *prevalence* package (Devleesschauwer et al., 2013) of R.

6.2.7 Multivariable logistic regression model

Multivariable logistic regression models were developed to identify and quantify risk factors for leptospirosis and Q fever in the PAZ adjusted population at the individual level. A multivariable mixed effects logistic regression model was used to account for the clustering of people within homesteads. The model was developed using *glmer* function in the *lme4* (Bates, 2014) package. The steps for model development were:

1. Univariable logistic regression was used to screen 45 variables against disease exposure. The variables used are listed in Appendix 11.
2. Variables were excluded from analysis if they were strongly correlated with another variable of interest to avoid multicollinearity problems and model estimate instability. Correlation analysis for categorical variables was performed by calculating the phi coefficient of correlation in the *psych* package (Revelle, 2014) in R. Variables with a phi coefficient >0.5 were excluded.
3. Variables with a *p*-value <0.2 in the univariable analysis were then used to develop a multivariable logistic regression model for each exposure.
4. A backwards stepwise approach was used for model selection by starting with a full model using all the predictors. Variables with the highest *p*-value were dropped in a stepwise fashion until the model with the lowest AIC was identified.
5. The ORs, CIs, and *p*-values were calculated from the model.

6.2.8 Diagnostic checking of multivariable mixed effects model

Traditional diagnostic procedures cannot account for the random effects in mixed effects models. Therefore a number of different approaches were used for model checking.

1. Variance Inflation Factors (VIFS) were calculated to check for collinearity. VIFS >4 were considered a problem and the variable removed from the model
2. Moran's I were calculated to check for spatial autocorrelation using the *ape* package (Paradis E., 2004)
3. The mixed effects models were simplified to the basic model that included only the disease outcome and the homestead. A second model was made using only the individual level factors and finally, the complete fitted multivariable mixed effect model. Median odds ratios (MOR) were calculated for the three models and compared (Merlo et al., 2005)

   $$MOR = exp\sqrt{2xV_A}x\ 0.6745$$

4. Percentage change in variance (PCV) was calculated between the three models (Merlo et al., 2005)

   $$PCV = \frac{V_A - V_B}{V_A}\ x\ 100$$

   V_A=variance in the empty model and V_B=variance of model with explanatory variables

6.3 Results

6.3.1 Demographics of the adjusted PAZ adjusted population

There were 416 homesteads recruited into the PAZ study, which included 2113 people and 983 cattle. For the purposes of this analysis, only the 980 participants aged over 20 years were included. The demographics of the adjusted PAZ adjusted population are presented in Table 6.1. The PAZ adjusted population was predominantly female (60.0%). Figure 6.2 shows the age profile of the PAZ adjusted population. The majority of the population was aged less than 50 years (72%). Over two thirds of the population were farmers. Almost 60% of the PAZ adjusted population owned cattle (Figure 6.3).

Variable	% population (95% CI) (n=980)
Gender	
Female	60.0 (56.3–63.8)
Male	40.0 (36.2–43.7)
Education	
Primary	80.3 (77.3–83.4)
Secondary	19.7 (16.6–22.7)
Occupation	
Farmer	69.1 (65.6–72.6)
Other	30.9 (27.4–34.4)
Knowledge of zoonoses	
Don't know	22.9 (19.7–26.1)
No	57.4 (53.6–61.2)
Yes	19.7 (16.6–22.7)

Table 6.1 Demographics of the People, Animals and their Zoonoses (PAZ) adjusted population

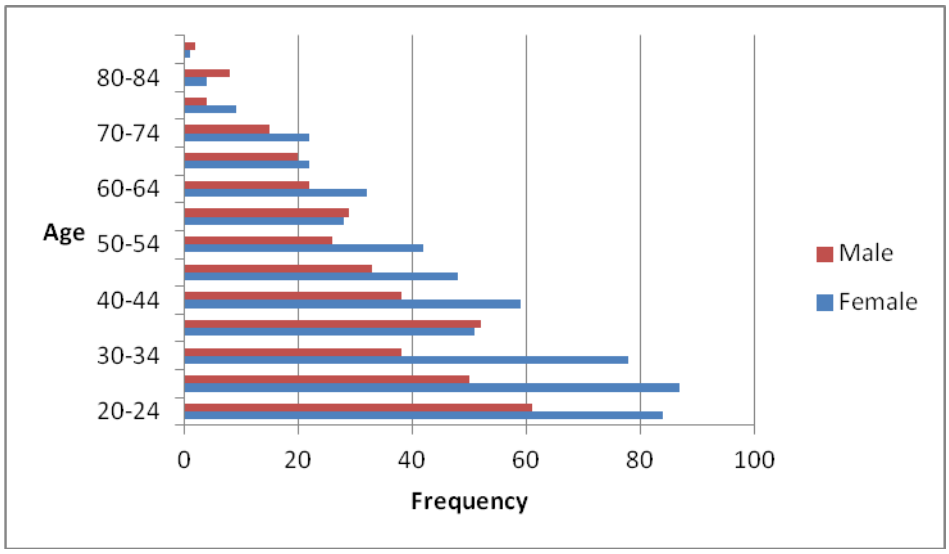


Figure 6.2 The age profile of the People, Animals and their Zoonoses (PAZ) adjusted population

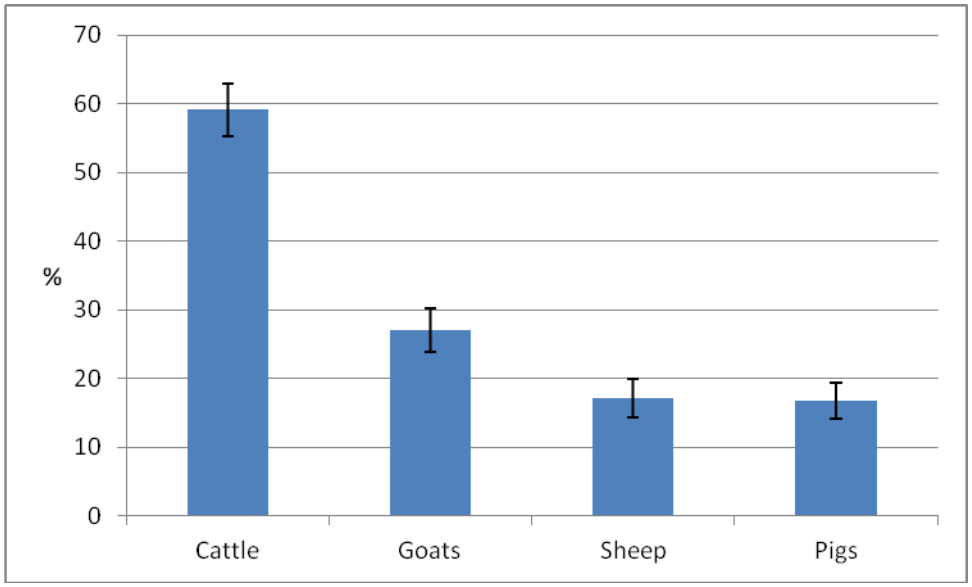


Figure 6.3 Animal ownership in the People, Animals and their Zoonoses (PAZ) adjusted population

6.3.2 Seroprevalence estimates

a. Leptospirosis

Table 6.2 shows the apparent prevalence for leptospirosis in the PAZ adjusted population, corrected for the design effect and the test sensitivity and specificity. The

apparent seroprevalence of leptospirosis was 6.5%. The estimates did not change after accounting for clustering, but did change after accounting for the test imperfections.

	Individual level apparent prevalence	Individual result adjusted for the design effect	Individual result adjusted for test se/sp†
Leptospirosis (n=951)	6.5 (5.1–8.3)	6.4 (4.5–8.2)	5.4 (3.8–7.1)

† se/sp sensitivity/specificity

Table 6.2 Seroprevalence estimates for leptospirosis in the People, Animals and their Zoonoses (PAZ) adjusted population

Figure 6.4 is a histogram of the Panbio units from the leptospirosis ELISA for the PAZ adjusted population. The red line indicates the negative cut-off and the blue line the positive cut-off. Equivocal results (between the lines) were considered negative for the purposes of this study. The histogram shows a large negative population. There is not a clear distinction between positive and negative results. Figure 6.5 shows the location of the leptospirosis positive homesteads. There is no spatial clustering evident.

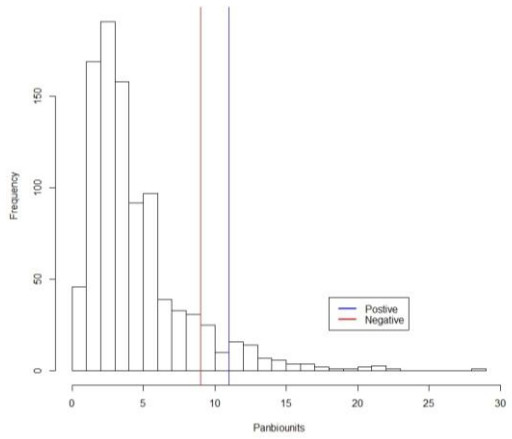


Figure 6.4 Histogram of the Panbio units for the People, Animals and their Zoonoses (PAZ) adjusted population

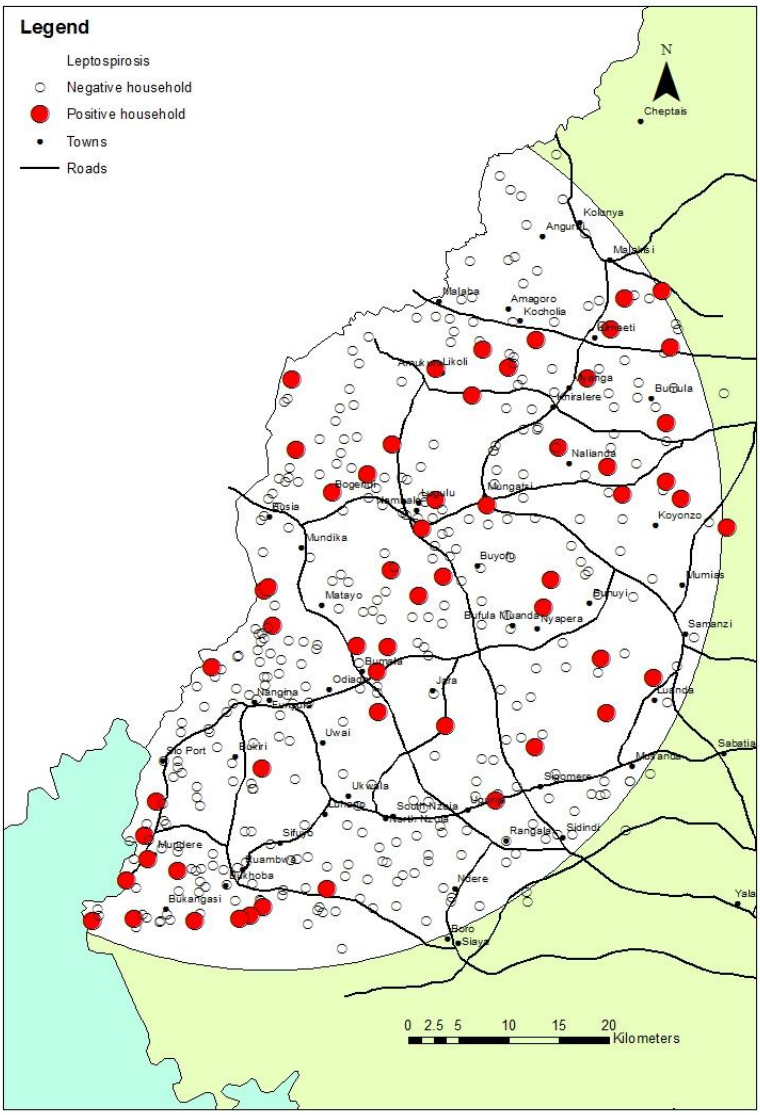


Figure 6.5 Map of the People, Animals and their Zoonoses (PAZ) adjusted population leptospirosis positive homesteads

b. Q fever

Table 6.3 shows the apparent prevalence Q fever in the PAZ adjusted population corrected for the design effect and the test sensitivity and specificity. The apparent seroprevalence of Q fever was 1.5%. The estimates did not change after accounting for clustering but did change after accounting for the test imperfections.

	Individual level apparent prevalence	Individual result adjusted for the design effect	Individual result adjusted for test se/sp†
Q fever (n=921)	1.5 (0.9–2.5)	1.6 (0.6–2.7)	0.4 (0–1.2)

† se/sp sensitivity/specificity

Table 6.3 Seroprevalence estimates for Q fever in the PAZ adjusted population

Figure 6.6 is a histogram of the Q fever corrected OD values. The red line indicates the negative cut-off and the blue line the positive cut-off. Equivocal results (between the lines) were considered negative for the purposes of this study. The histogram shows a large negative population. There is an extremely small positive population.

Figure 6.7 shows the location of the Q fever positive homesteads. These homesteads are clustered in the south of the study area.

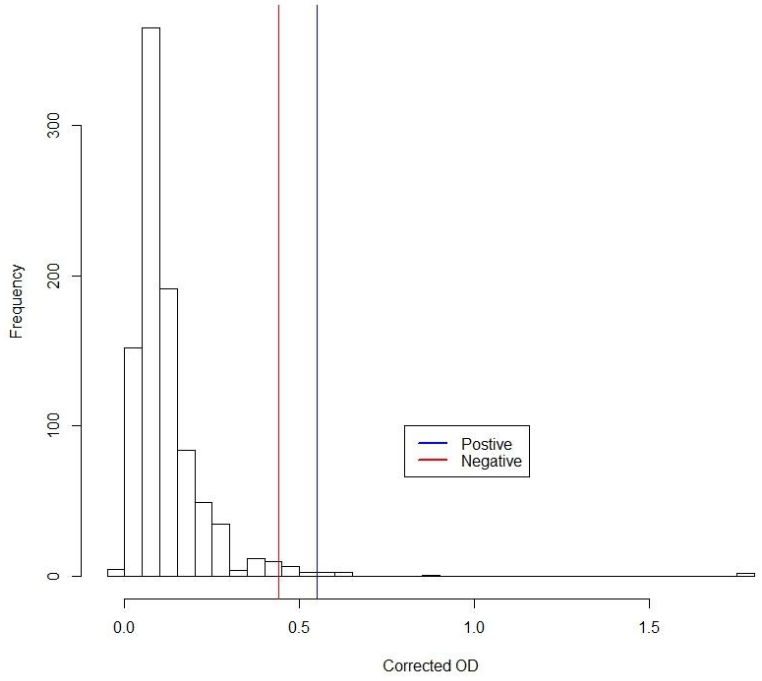


Figure 6.6 Histogram of the corrected optical density (OD) values for Q fever

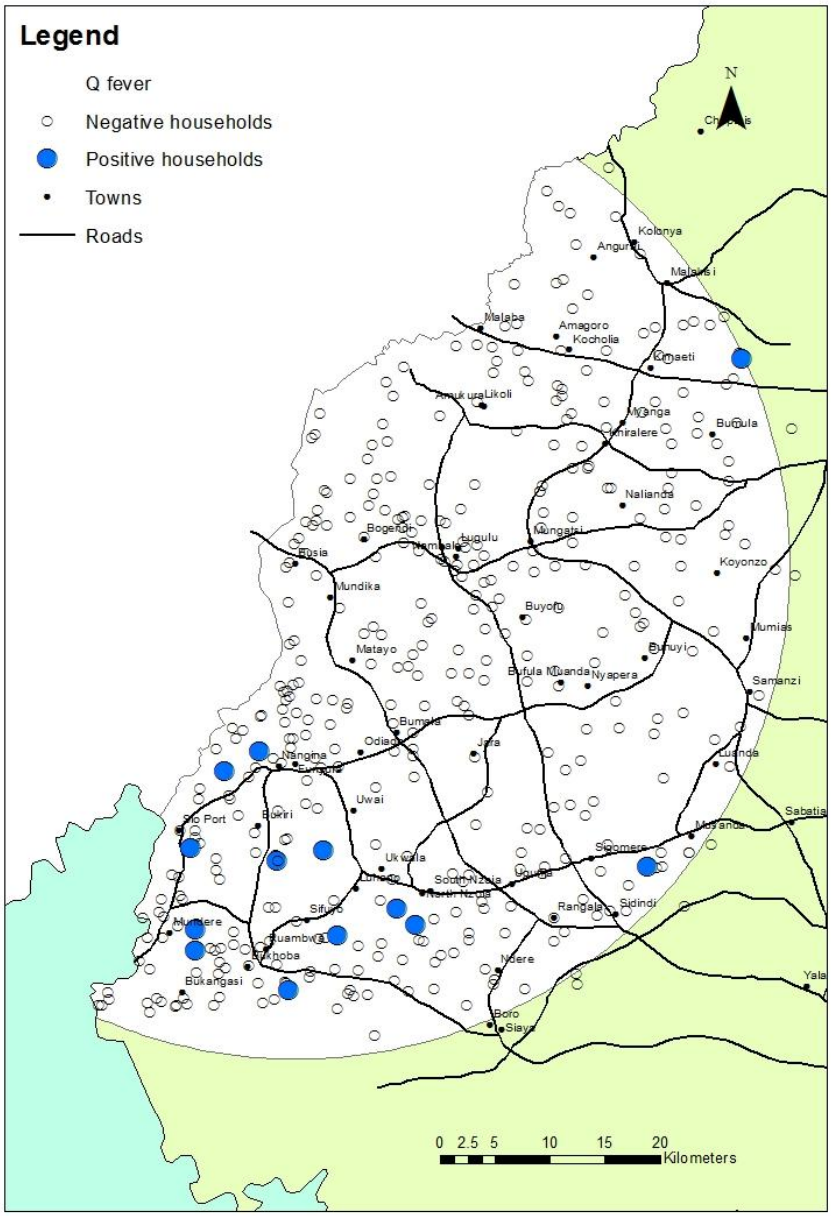


Figure 6.7 Map of the People, Animals and their Zoonoses (PAZ) adjusted population Q fever positive homesteads

6.3.3 Leptospirosis

a. Univariable logistic regression

The univariable logistic regression analysis identified 9 variables with *p*-value <0.2 (Table 6.4). The variables that were significantly associated with leptospirosis seropositivity include: milking cattle (OR 2.0); milking goats or sheep (OR 4.5); attending to animal births (OR 2.1); and being currently infected with *Schistosoma mansoni* (OR 2.7).

Figure 6.8 is a correlation matrix between these variables. There was a medium correlation between gender and milking cattle but no highly correlated variables.

Variable	% population (n)	% positive (n)	OR (95% CI)	*p*–value
Gender				
Female	59.2 (564)	5.3 (30)	1	Ref
Male	40.8 (389)	8.2 (32)	1.6 (0.9–2.9)	0.107
Hunting				
No	96.0 (914)	6.2 (57)	1	Ref
Yes	4.0 (38)	13.2 (5)	2.4 (0.8–7.4)	0.117
Milking cattle				
No	66.4 (631)	5.1 (32)	1	Ref
Yes	33.6 (320)	9.4 (30)	2.0 (1.1–3.5)	**0.024**
Milking goats or sheep				
No	97.2 (924)	6.1 (56)	1	Ref
Yes	2.8 (27)	22.2 (6)	4.5 (1.6–12.4)	**0.004**
Assisting animal births				
No	86.0 (818)	5.7 (47)	1	Ref
Yes	14.0 (133)	11.3 (15)	2.1 (1.1–4.3)	**0.031**
Borehole				
No	60.4 (576)	7.6 (44)	1	Ref
Yes	39.6 (377)	4.8 (18)	0.6 (0.3–1.1)	0.120
Spring				
No	63.9 (609)	5.3 (32)	1	Ref
Yes	36.1 (344)	8.7 (30)	1.7 (1.0–2.9)	**0.039**
S. mansoni				
No	93.2 (861)	6.0 (52)	1	Ref
Yes	6.8 (63)	14.3 (9)	2.7 (1.2–6.3)	0.021
HIV				
No	89.2 (850)	6.9 (59)	1	Ref
Yes	10.8 (103)	2.9 (3)	0.4 (0.1–1.5)	0.179

Table 6.4 Odds ratios for leptospirosis in People, Animals and their Zoonoses (PAZ) adjusted population.

	Gender	Hunting	Milking cattle	Milking goat/sheep	Animal births	*S. mansoni*	HIV	Spring	Borehole
Gender		0.07	**0.46**	-0.02	0.03	0.01	-0.11	0.33	-0.18
Hunting			0.07	0.08	0.14	0.05	-0.04	-0.03	-0.05
Milking cattle				0.07	0.14	-0.01	-0.03	**0.43**	**-0.44**
Milking goats/sheep					0.13	0.08	-0.02	-0.05	-0.04
Animal births						0.1	-0.03	-0.05	-0.12
S. mansoni							-0.03	-0.09	-0.1
HIV								-0.02	0.03
Spring									-0.29
Borehole									

Figure 6.8 Correlation matrix for variables for exposure to leptospirosis in People, Animals and their Zoonoses (PAZ) adjusted population

b. Multivariable model selection

The multivariable model was developed with the 9 selected variables. Table 6.5 demonstrates the models that were created by the backward stepwise selection. The final model is highlighted in bold and has an AIC of 437.0. The last model in the table is one step past the model with the best fit.

Model	AIC
leptoresult ~ gender + hunting + milking_shoats* + animal_births + milking_cattle +Smansoni + HIV_Result + home_source_water_wet_borehole + home_source_water_wet_spring + (1 | homestead_id) ,family=binomial(logit),data=pazadultlepto	441.2
leptoresult ~ sex + hunting + milking_shoats+ animal_births + milking_cattle + Smansoni + HIV_Result + home_source_water_wet_spring + (1 | homestead_id) ,family=binomial(logit),data=pazadultlepto	439.4
leptoresult ~ hunting1 + milking_shoats + animal_births + milking_cattle + Smansoni + HIV_Result + home_source_water_wet_spring + (1 | homestead_id) ,family=binomial(logit),data=pazadultlepto	438.0
leptoresult ~ hunting1 + milking_shoats + animal_births + milking_cattle + Smansoni + home_source_water_wet_spring + (1 | homestead_id) ,family=binomial(logit),data=pazadultlepto	437.5
leptoresult ~ hunting1 + milking_shoats + milking_cattle + Smansoni + home_source_water_wet_spring + (1 | homestead_id) ,family=binomial(logit),data=pazadultlepto	437.2
leptoresult ~ milking_shoats + milking_cattle + Smansoni + home_source_water_wet_spring + (1 | homestead_id) ,family=binomial(logit),data=pazadultlepto	**437.0**
leptoresult ~ milking_shoats + Smansoni + home_source_water_wet_spring + (1 | homestead_id) ,family=binomial(logit),data=pazadultlepto	439.4

*Shoats – colloquial term for goats and sheep

Table 6.5 Model selection for leptospirosis in the People, Animals and their Zoonoses (PAZ) adjusted population.

c. Final multivariable model

The results of the final multivariable logistic regression model are indicated in Table 6.6. The risk factors significantly associated with leptospirosis exposure in the PAZ adjusted population were: milking cattle; milking sheep/goats; being infected with *S. mansoni*; and having a spring as a water source.

Variables	OR (95% CI)	*p*-value	VIFS
Milking cattle	1.8 (1.0–3.1)	0.034	1.036
Milking goats/sheep	4.1 (1.5–11.2)	0.005	1.062
S. mansoni	3.2 (1.4–7.0)	0.004	1.037
Water source – spring	2.1 (1.2–3.6)	0.009	1.068

Table 6.6 Results of multivariable logistic regression for exposure to leptospirosis in the People, Animals and their Zoonoses (PAZ) adjusted population

d. Model checking

The Morans I measure for spatial autocorrelation was not significant (p=0.40) showing that there is no spatial autocorrelation in the model.

Table 6.7 shows that the mixed model has the lowest AIC and a median OR of 1 indicating that the clustered design did not affect the results.

Variable	Empty model	Model with individual variables	Model with individual and slaughterhouse variables
Individual variables			
Milking cattle		1.9 (1.1–3.2)*	1.8 (1.0–3.1)*
Milking sheep/goats		3.4 (1.3–9.0)*	4.1 (1.5–11.2)**
S. mansoni		2.7 (1.2–5.8)*	3.2 (1.4–7.0)**
Homestead variables			
Water source – spring			2.1 (1.2–3.6)**
Variance (SE)	0.793	$6.5x10^{-11}$	$8.0x10^{-11}$
Proportional change in variance	Reference	100%	100%
Median OR	1.18	1.00	1.00
AIC	462.0	442.0	437.0

*p <0.05, **p < 0.01, and ***p < 0.001

Table 6.7 Individual and homestead level predictors associated with leptospirosis in the People, Animals and their Zoonoses (PAZ) adjusted population by multivariable logistic regression

6.3.3 Q fever

a. Univariable logistic regression

The univariable logistic regression analysis identified 3 variables with *p* values <0.2 (Table 6.8). The only variable that was significantly associated with Q fever seropositivity was the making of manure. Figure 6.9 is a correlation matrix between these variables.

Variable	% population (n)	% positive (n)	OR (95% CI)	*p*-value
Occupation				
Other	32.6 (297)	1.0 (3)	1	Ref
Farmer	67.4 (613)	1.8 (11)	16.7 (0.7–376)	0.076
Manure				
No	37.4 (344)	1.4 (5)	1	Ref
Yes	62.6 (576)	1.6 (9)	23.4 (1.1–48.2)	**0.041**
Wounds				
No	97.5 (898)	12 (1.3)	1	Ref
Yes	2.5 (23)	2 (8.7)	10.6 (0.4–27.0)	0.153

Table 6.8 Odd ratios for Q fever in People, Animals and their Zoonoses (PAZ) adjusted population.

	Farmer	Manure	Wounds
Farmer		0.1	-0.06
Manure			0.01
Wounds			

Figure 6.9 Correlation matrix for variables for exposure to Q fever in People, Animals and their Zoonoses (PAZ) adjusted population

b. Multivariable model selection

The multivariable model was developed with the selected 3 variables. Table 6.9 demonstrates the models that were created by the backward stepwise selection. The final model is highlighted in bold with an AIC of 99.35. The last model in the table is one step past the model with the best fit.

Model	AIC
qfever ~ occupation + wounds + animal_manure + (1 | homestead_id) ,family=binomial(logit),data=pazadultlepto	99.38
qfever ~ occupation + animal_manure +(1 | homestead_id) ,family=binomial(logit),data=pazadultlepto	**99.35**
qfever ~ animal_manure1+ (1 | homestead_id) ,family=binomial(logit),data=pazadultlepto)	101.20

Table 6.9 Model selection for Q fever in People, Animals and their Zoonoses (PAZ) adjusted population

c. Final multivariable model

The results of the final multivariable logistic regression model are indicated in Table 6.10. There were no risk factors identified as significantly associated with Q fever exposure in the PAZ adjusted population.

Variables	OR (95% CI)	*p*-value	VIFS
Occupation – farmer	14.2 (0.4–472)	0.137	1.057
Manure	15.0 (0.5–416)	0.109	1.057

Table 6.10 Results of multivariable logistic regression for exposure to Q fever in the People, Animals and their Zoonoses (PAZ) adjusted population

d. Model checking

The Morans I measure for spatial autocorrelation was not significant (p=0.16) showing that there is no spatial autocorrelation in the model.

The median ORs were not calculated, as the model did not include any homestead level predictors.

6.4 Discussion

The PAZ adjusted population was predominantly young and female. A large proportion (80.3%) of the PAZ adjusted population had primary level education which is consistent with the report of the Kenya Demographic and Health Survey for the region (KNBS, 2010). The majority of the population were farmers (69.1%) which has also been reported elsewhere (Adazu et al., 2005). There was a paucity of knowledge regarding zoonotic disease with only 20% of people being aware of zoonoses. A large number of the population had contact with livestock, which is a risk factor for zoonotic diseases.

6.4.1 Leptospirosis

The apparent prevalence of leptospirosis in the PAZ adjusted population was 6.5%. The clustered design of the sample did not affect the prevalence markedly. Adjustments for the test sensitivity and specificity showed the true prevalence to be 5.4%. The ELISA output shows a large negative population but there is an unclear distinction between the positive and negative populations (Figure 6.4). As discussed in previous chapters regarding the slaughterhouse worker results the ELISA needs to be validated in the Kenyan setting. There was no apparent spatial clustering of the leptospirosis positive homesteads (Figure 6.5).

The results of the multivariable logistic regression model for leptospirosis in the adjusted PAZ adjusted population indicated a number of risk factors for exposure, which will be discussed individually (Figure 6.10).

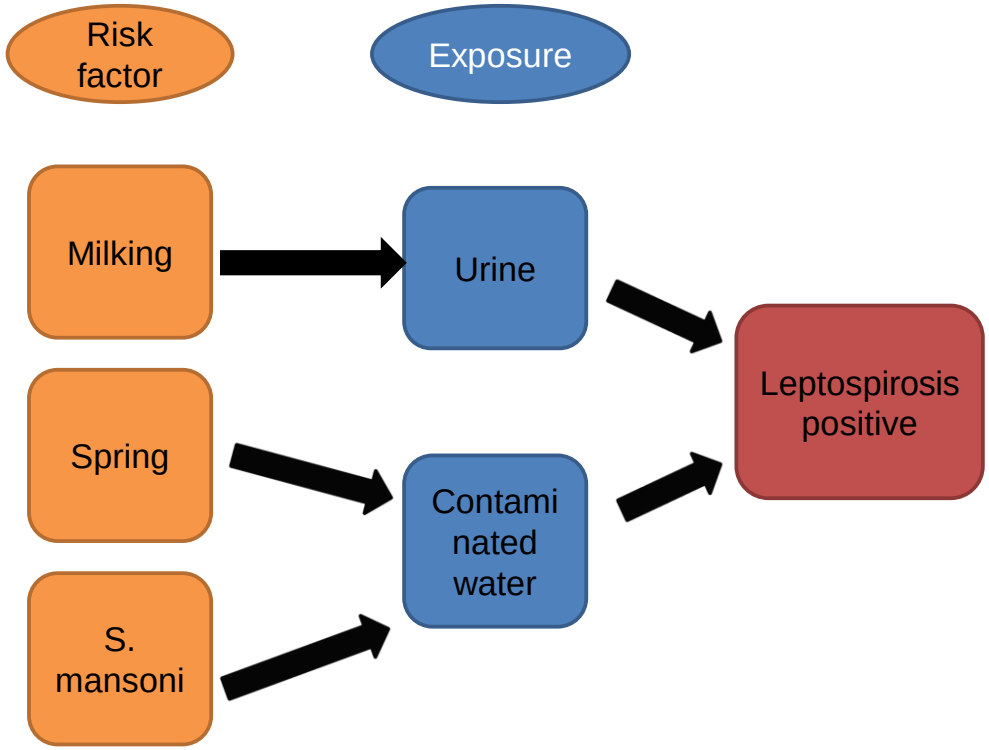


Figure 6.10 Causal framework of risk factors for leptospirosis in the People, Animals and their Zoonoses (PAZ) adjusted population

Contact with infected animals is a risk factor for leptospirosis transmission. Milking cattle (OR 1.8; 95% CI 1.0–3.1) and milking goats /sheep (OR 4.1; 95% CI 1.5–11.2) were identified as risk factors for leptospirosis exposure in the PAZ adjusted population. Milking is a known risk factor for leptospirosis due to direct contact with animal urine (Waitkins, 1986, Hart et al., 1984)

Using spring water in the homestead was associated with leptospirosis exposure (OR 2.1: 95% CI 1.2–3.6). Leptospirosis from contaminated water sources, particularly springs (fountains) is documented in the literature (Cacciapuoti et al., 1987, Levett, 2001). There was no single point source of infection related to exposure in this context.

Infection with *S. mansoni* was identified as a risk factor for leptospirosis in the PAZ adjusted population (OR 3.2; 95% CI 1.4–7.0). This result is likely indicative of a co-infection due to exposure to the same water source. *S. mansoni* is transmitted through contact with infected water (WHO, 2014b), and leptospirosis can be transmitted from contaminated water though cuts (Faine, 1999). The southern part of the study area bordering Lake Victoria is a high risk area for *S. mansoni* (Handzel et al., 2003). This area is prone to flooding, and people use the lake for fishing, swimming, and collecting water, which are risk factors for leptospirosis (Jackson et al., 1993, Christova et al., 2003).

6.4.2 Q fever

The apparent prevalence of Q fever was 1.5%. There was no effect on the prevalence from the clustered design of the sample. However the true prevalence was reduced to 0.4% after adjusting for the test sensitivity and specificity. There were an extremely small number of positive samples evident from the ELISA output with only a couple of samples having a clear distinction from the negative population (Figure 6.6). The Q fever cases are clustered in the southern part of the study area (Figure 6.7). This area also has the greatest cattle population density (Figure 6.1).

The results of the multivariable logistic regression model for Q fever in the PAZ adjusted population did not show any significant variables for seropositivity. The two variables in the model were: being a farmer (OR 14.2; 95% CI 0.4–472); and having contact with manure (OR 15.0; 95% CI 0.5–416). Both variables had extremely wide confidence intervals. The wide intervals suggest a large amount of uncertainty regarding these results. In order to verify these interactions further study would need

to be done investigating Q fever in this community, particularly focusing on the identified high risk areas. Although the results are not precise, they are not insensible (Figure 6.11). Being a farmer is an established risk factor for Q fever (Maurin and Raoult, 1999). Contaminated goat manure has been shown to be a source of Q fever infection in other areas (Hermans et al., 2014).

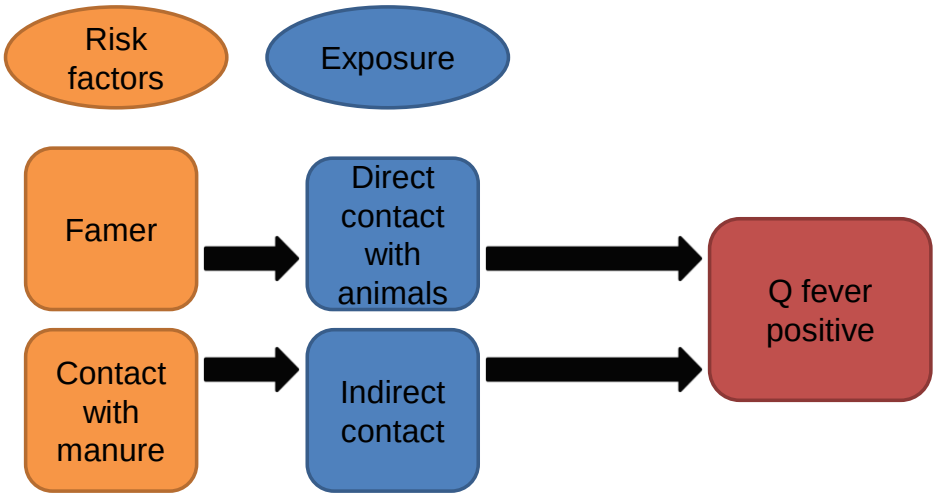


Figure 6.11 Causal framework of risk factors for Q fever in the People, Animals and their Zoonoses (PAZ) adjusted population

6.5 Conclusion

This study reports the seroprevalence of leptospirosis and Q fever in the community of western Kenya. The study findings suggest that the level of zoonotic disease awareness in the community is very low. The hypothesis for this study was that people who have contact with animals are more at risk of zoonotic disease.

The hypothesis that contact with animals is a risk factor for leptospirosis and Q fever is true, but the study identified other factors related to exposure. Leptospirosis seropositivity in the community was associated with contact with cattle, goats, and sheep through milking which supports the original hypothesis. However exposure to contaminated water sources (springs as a source of water as well as Lake Victoria) were also risk factors for leptospirosis seropositivity in this community. Indirect contact with animals was a risk factor for Q fever seropositivity (contact with animal manure).

It is clearly evident from the results described in this chapter that the risk factors for zoonotic disease in the PAZ adjusted population were predominantly contact with animals and animal products. However the epidemiology of leptospirosis was complicated by contact with contaminated water sources.

Further investigation of high risk areas for zoonotic disease and targeted control programmes for these groups would be advised.

Chapter 7

Comparing the risks for exposure to leptospirosis and Q fever between slaughterhouse workers and the community

7.1 Introduction

Slaughterhouse workers, veterinarians, and farmers are often cited as high risk groups for zoonotic disease but this is rarely quantified by comparison to the general population (Chan et al., 1987, Schoonman and Swai, 2009, Sharma et al., 2006).

The hypotheses of this study were:

1. slaughterhouse workers are healthier than the general population
2. slaughterhouse workers are more aware of zoonoses than the general population
3. slaughterhouse workers are more exposed to zoonotic disease than the community
4. the geospatial zoonotic disease risk in slaughterhouse workers resembles that in the human and cattle population of the study area

This chapter compares health indices between slaughterhouse workers and the PAZ adjusted population to determine if slaughterhouse workers are more or less healthy than the general population. The chapter also reports the seroprevalence and the quantified risk of leptospirosis and Q fever in slaughterhouse workers as compared to that of the concomitant general population to determine if slaughterhouse workers are more exposed to zoonotic disease. Finally, the spatial distribution of exposure to these pathogens is reported and compared for slaughterhouse workers and the PAZ adjusted population.

7.2 Methods

7.2.1 Sampling frame

The data used for this paper incorporates information from the slaughterhouse worker sample. This sample has been extensively described in previous chapters and will not be repeated here unless referred to for comparison. Data from the PAZ study (Doble and Fevre, 2010) described in Section 6.2 are included for comparison. The PAZ adjusted population consisted of only individuals aged over 20.

In addition, disease data regarding Q fever in cattle in the study area are included in order to geospatially compare with disease in people.

Cattle were sampled at recruited homesteads. The person responsible for the animals was asked information on the health and parity of each animal and the animals were examined by an animal health technician or veterinarian assigned to the project and data recorded for demeanour, coat condition, body condition, and presence of external parasites.

24mls of blood was collected from the jugular vein into two 10ml plain and a 4ml EDTA BD Vaccutainers®. All samples were transported in a cool box to the ILRI laboratory in Busia for analysis.

Animal blood samples were prepared in the Busia laboratory. Plain blood tubes were centrifuged for 20 minutes at 3000 rpm and the sera aliquoted into Nalgene® 2 ml cryovials and kept frozen at −40°C. Further serological testing was performed at the ILRI Nairobi laboratory for Q fever using the CHEKIT Q Fever Antibody ELISA Test Kit (IDEXX Laboratories, Wetherby, UK). This assay detects antibodies to *C.*

burnetii in ruminant serum, plasma and milk samples. Sera were prediluted 1:400 using CHECKIT wash solution. 100µl of diluted samples and controls were dispensed into wells of a precoated microtitre plate and incubated at 37ºC for 60 minutes. The plate was washed with approximately 300µl CHEKIT wash solution 3 times. 100µl of conjugate was added to each well and incubated at 37ºC for 60 minutes in a humid chamber. The plate was washed with approximately 300µl CHEKIT wash solution 3 times. 100µl of TMB substrate was added to each well and the plate incubated at room temperature for 15 minutes. 100µl of stop solution was added to each well and the results read at a wavelength of 450nm. The OD results of duplicate samples were averaged and the following equation applied to the results:

$$Value\ \% = \frac{OD_{sample} - OD_{neg}}{OD_{pos} - OD_{neg}}\ x\ 100\%$$

Values less than 30% were considered negative; and values greater than or equal to 40% were considered positive with values in between considered equivocal. The CHECKIT Q fever ELISA is reported by the manufacturer to have 100% sensitivity and 100% specificity.

7.2.2 Data management

Microsoft® Access databases were used to manage data.

7.2.3 Variable selection

Variables were selected from both the PAZ individual and the slaughterhouse individual datasets. Variables were incorporated into a combined dataset for analysis.

The criteria for inclusion were that the questions had to be asked in the same manner and coded or recoded using the same methodology. Variables selected included:

- knowledge of zoonoses – how many participants knew that animals could be a source of human disease
- Level of education
- Smoking behaviour
- Latrine use
- Animal ownership
- HIV
- Parasitic condition tested
- Reported health conditions
- Use of medicines

7.2.4 Data analysis

A combined dataset was created in R using matching variables from the slaughterhouse workers' dataset and the PAZ dataset. Univariable logistic regression was used to compare health indices between the two datasets in the *lme4* package. (Bates, 2014). Univariable logistic regression was used to compare prevalence estimates between slaughterhouse workers and the PAZ adjusted population in the *lme4* package. A univariable mixed effects logistic regression model was used to account for the clustering of people within homesteads and slaughterhouses. Age and gender were added as fixed effects to the multivariable mixed effects model as these factors were expected to confound the results.

7.2.4 Spatial analysis

For mapping purposes slaughterhouses and homesteads were considered positive for each pathogen if one or more inhabitants were positive for leptospirosis or Q fever respectively. Kernel smoothing was used to assess the density of positive slaughterhouses and homesteads using the *sparr* package (Davies et al., 2011) in R with a fixed bandwidth of 5km and correction for edge effects. The kernel intensity of seropositive slaughterhouses/homesteads was divided by the kernel intensity of the seronegative slaughterhouses/homesteads in the study area creating a "relative risk" surface. This technique does not assess clustering as conducted in Chapter 4 but produces spatially smooth risk maps that allow areas where the greatest relative risk for seropositivity to be identified.

7.3 Results

7.3.1 Comparison between slaughterhouse workers and the PAZ adjusted population

a. Knowledge and risk behaviours

Univariable logistic regression analysis examined the difference in knowledge, risk behaviours, and animal contacts between slaughterhouse workers and the PAZ adjusted population. The differences in knowledge and risk behaviours between slaughterhouse workers and the PAZ adjusted population are presented in Table 7.1. Slaughterhouse workers were less educated with 0.3 times the odds of having secondary education than the PAZ adjusted population. Slaughterhouse workers were more likely to smoke (OR 2.3). Slaughterhouse workers were more likely to use the latrine every time they defecated (OR 3.8).

	Apparent prevalence % (n)	**OR (95% CI)**	***p*–value**
Zoonoses awareness			
PAZ	20.1 (196)	1	Ref
SHW	31.2 (230)	1.2 (0.9–1.7)	0.154
Secondary education			
PAZ	20.3 (199)	1	Ref
SHW	14.9 (110)	0.3 (0.2–0.5)	**<0.001**
Smoking regularly			
PAZ	7.2 (70)	1	Ref
SHW	23.4 (173)	2.3 (1.6–3.2)	**<0.001**
Use latrine every time			
PAZ	86.9 (849)	1	Ref
SHW	81.1 (569)	3.8 (2.0–7.0)	**<0.001**

Table 7.1 Comparison of knowledge and risk behaviours between slaughterhouse workers and People, Animals and their Zoonoses (PAZ) adjusted population

b. Animal contacts

Table 7.2 presents the univariable logistic regression results for animal contacts between slaughterhouse workers and the PAZ adjusted population. Both slaughterhouse workers and the PAZ adjusted population were equally likely to own cattle (OR 1.0). Slaughterhouse workers were less likely to own sheep (OR 0.4) but more likely to own goats (OR 2.3) and pigs (OR 1.6).

	Apparent prevalence % (n)	**OR (95% CI)**	***p*–value**
Own cattle			
PAZ	63.3 (620)	1	Ref
SHW	65.9 (486)	1.0 (0.5–2.4)	0.928
Own sheep			
PAZ	32.2 (316)	1	Ref
SHW	15.3 (113)	0.4 (0.3–0.5)	**<0.001**
Own goats			
PAZ	20.0 (196)	1	Ref
SHW	26.6 (196)	2.3 (1.8–2.9)	**<0.001**
Own pigs			
PAZ	20.9 (205)	1	Ref
SHW	30.1 (222)	1.6 (1.3–2.0)	**<0.001**
Dog contact			
PAZ	75.3 (738)	1	Ref
SHW	76.4 (564)	1.0 (0.6–1.6)	0.990

Table 7.2 Comparison of animal ownership between slaughterhouse workers and People, Animals and their Zoonoses (PAZ) adjusted population

C. Diagnosed disease

Table 7.3 presents the results of the univariable logistic regression comparing selected health indices between slaughterhouse workers and the PAZ adjusted population. Slaughterhouse workers had 1.8 times the odds of having HIV at the time of interview than the PAZ adjusted population. Slaughterhouse workers were more likely to have malaria (OR 1.5). Slaughterhouse workers had increased odds of having a wound at the time of the interview (OR 2.4) (Table 7.3).

	Apparent prevalence % (n)	**OR (95% CI)**	***p*–value**
HIV			
PAZ	10.9 (106)	1	
SHW	12.1 (89)	1.8 (1.1–2.9)	**0.020**
Malaria at time of interview			
PAZ	11.0 (108)	1	
SHW	15.2 (110)	1.5(1.1–2.3)	**0.056**
Wounds			
PAZ	2.7 (26)	1	
SHW	7.7 (57)	2.4 (1.3–4.7)	**0.008**
Entamoeba histolytica			
PAZ	28.6 (270)	1	
SHW	15.7 (114)	0.6 (0.4–0.9)	**0.004**
Hookworm			
PAZ	35.2 (332)	1	
SHW	30.7 (223)	0.8 (0.6–1.1)	0.111
Schistosoma mansoni			
PAZ	8.6 (81)	1	
SHW	3.7 (27)	0.3 (0.008–21.3)	0.542

Table 7.3 Comparison of select diagnosed diseases and clinical symptoms between slaughterhouse workers and the People, Animals and their Zoonoses (PAZ) adjusted population

d. Self reported disease episodes

Slaughterhouse workers had reduced odds for backache (OR 0.7); headache (OR 0.7); diarrhoea (OR 0.7); and abdominal pain (OR 0.6) (Table 7.4). However they had increased odds for having a cough (OR 1.4). Slaughterhouse workers had higher odds for taking medication in the past 3 months: antibiotic (OR 2.4); anti-inflammatory (OR 2.1); and anti-malarials (OR 3.8) (Table 7.5).

	Prevalence % (n)	OR (95% CI)	*p*–value
Cough			
PAZ	45.8 (449)	1	
SHW	50.0 (369)	1.4 (1.1–1.8)	**0.008**
Fever			
PAZ	61.7 (605)	1	
SHW	62.6 (462)	1.3(1.0–1.6)	0.060
Joint pain			
PAZ	61.5 (603)	1	
SHW	53.4 (394)	0.9 (0.7–1.1)	0.258
Backache			
PAZ	66.6 (653)	1	
SHW	47.8 (353)	0.7 (0.5–0.9)	**0.005**
Headache			
PAZ	76.6 (751)	1	
SHW	61.8 (456)	0.7(0.6–0.9)	**0.022**
Diarrhoea			
PAZ	29.0 (283)	1	
SHW	21.5 (159)	0.7(0.5–0.9)	**0.032**
Weight loss			
PAZ	15.7 (154)	1	
SHW	12.9 (95)	0.9 (0.6–1.3)	0.586
Abdominal pain			
PAZ	61.1 (599)	1	
SHW	41.6 (307)	0.6 (0.4–0.7)	**<0.001**

Table 7.4 Comparison of self reported symptoms between slaughterhouse workers and People, Animals and their Zoonoses (PAZ) adjusted population

	Apparent prevalence % (n)	OR (95% CI)	*p*-value
Antibiotic			
PAZ	10.2 (102)	1	
SHW	17.2 (127)	2.4 (1.6–3.5)	**<0.001**
Anti-inflammatory			
PAZ	33.1 (329)	1	
SHW	45.4 (335)	2.1 (1.6–2.7)	**<0.001**
Anti-malarial			
PAZ	9.8 (98)	1	
SHW	23.3 (172)	3.8 (2.6–5.7)	**<0.001**

Table 7.5 Comparison of recent medications between slaughterhouse workers and People, Animals and their Zoonoses (PAZ) adjusted population

e. Leptospirosis seroprevalence

The apparent seroprevalence of leptospirosis in the slaughterhouse workers was 13.4% and in the PAZ adjusted population was 6.5%. The apparent and adjusted seroprevalence estimates are indicated in Table 7.6. The OR for exposure to leptospirosis in slaughterhouse workers compared with the PAZ adjusted population was 2.3 (95% CI 1.6–3.4; p <0.001).

	Apparent prevalence	Prevalence adjusted for clustering	Prevalence adjusted for tests	OR	*p*–value
Leptospirosis					
Slaughterhouse workers n=737	13.4 (11.1–16.1)	13.6 (10.9–16.4)	12.7 (10.2–15.4)	2.3 (1.6–3.4)	<0.001
PAZ people n=951	6.5 (5.1–8.3)	6.4 (4.5–8.2)	5.4 (3.8 –7.1)	1	

Table 7.6 Prevalence of leptospirosis in slaughterhouse workers and the People, Animals and their Zoonoses (PAZ) adjusted population

f. Q fever seroprevalence

The apparent seroprevalence of Q fever in slaughterhouse workers was 4.5% and in the PAZ adjusted population was 1.5%. The apparent and adjusted seroprevalence estimates are indicated in Table 7.7. The prevalence of Q fever was reduced in both groups after accounting for the sensitivity and specificity of the tests. The OR for exposure to Q fever in slaughterhouse workers compared with the PAZ adjusted population was 1.9 (95% CI 1.0–3.8; p=0.060).

	Apparent prevalence	**Prevalence adjusted for clustering**	**Prevalence adjusted for tests**	**OR**	***p*–value**
Q fever					
Slaughterhouse workers n=737	4.5 (3.2–6.2)	4.6 (3.1–6.1)	3.4 (1.9–5.2)	1.9 (1.0–3.8)	0.060
PAZ people n=921	1.5 (0.9–2.5)	1.6 (0.6–2.7)	0.4 (0–1.3)	1	

Table 7.7 Prevalence of Q fever in slaughterhouse workers and the People, Animals and their Zoonoses (PAZ) adjusted population

7.3.2 Spatial analysis

The following maps show the spatial risk for leptospirosis and Q fever in slaughterhouse workers and the PAZ adjusted sample.

a. Leptospirosis

The results of the kernel density mapping for leptospirosis in slaughterhouses and the PAZ adjusted population demonstrate spatial heterogeneity but do not appear to correlate with one another (Figures 7.1). Areas of greatest relative risk for leptospirosis seropositivity in slaughterhouse workers appear to be in the central and eastern part of the study area. These contrasts to the PAZ adjusted population where the areas of greatest relative risk for seropositivity were in the north-eastern part of the study area and a small area in the south of the study area around Lake Victoria.

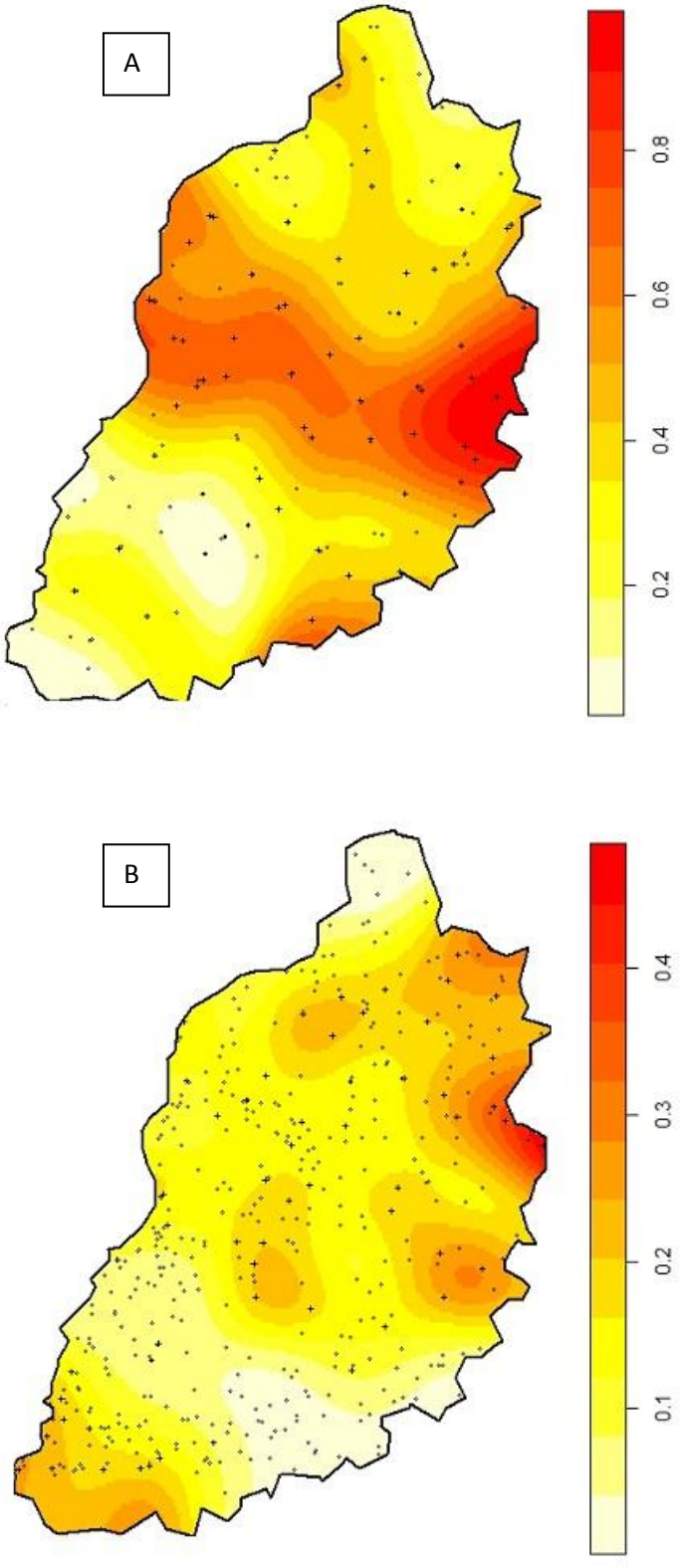


Figure 7.1 Spatially smoothed relative risk for leptospirosis

A) slaughterhouse, B) homesteads

b. Q fever

The results of the Kernel density mapping for Q fever in slaughterhouse and the PAZ adjusted population are demonstrated in Figure 7.2. The results of the Kernel density mapping for Q fever in cattle are demonstrated in Figure 7.3. Spatial heterogeneity is demonstrated for slaughterhouse workers, the PAZ adjusted population and cattle. Areas identified of greatest relative risk for Q fever seropositivity in slaughterhouses were in the eastern-central study area. This contrast to the PAZ adjusted population, where the area of greatest relative risk was in the south of the study area. The area of greatest relative risk for Q fever in cattle were also in the south of the study area.

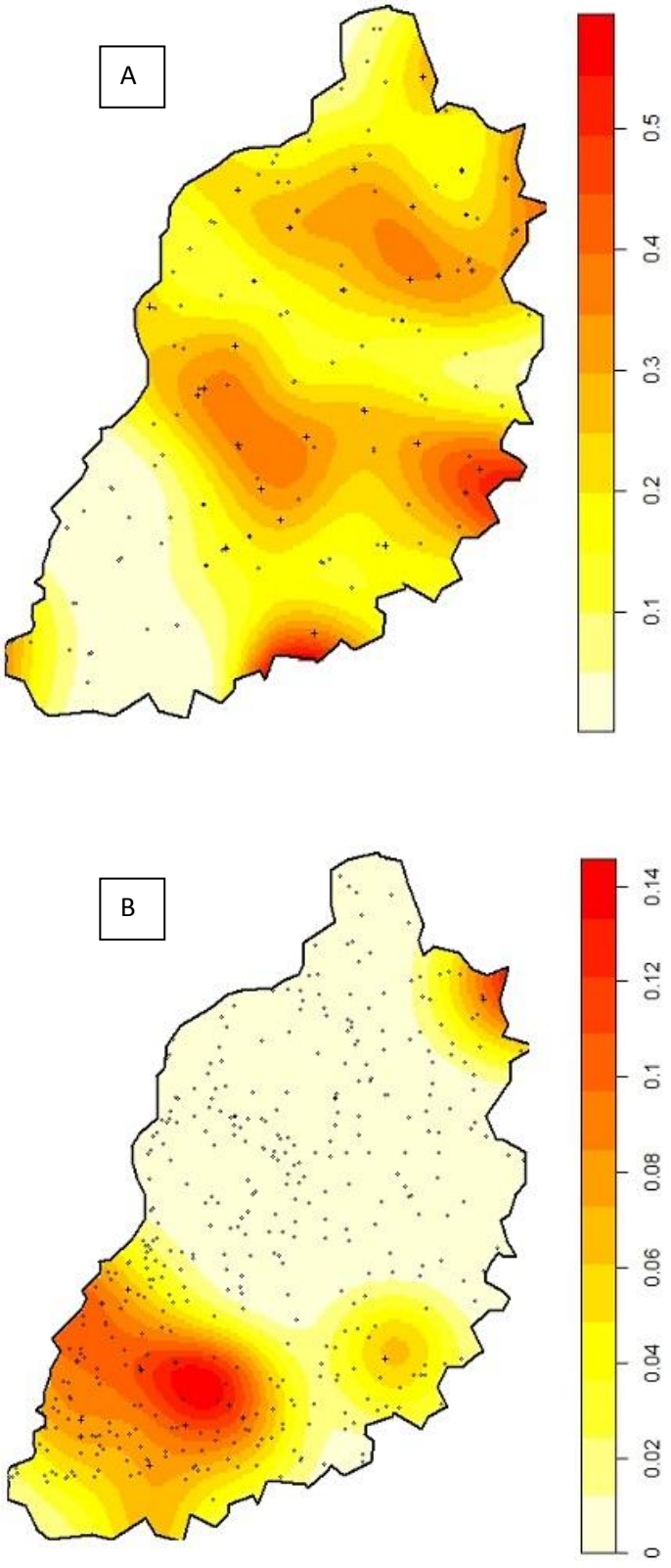


Figure 7.2 Spatially smoothed relative risk for Q fever A) slaughterhouses, B) homesteads

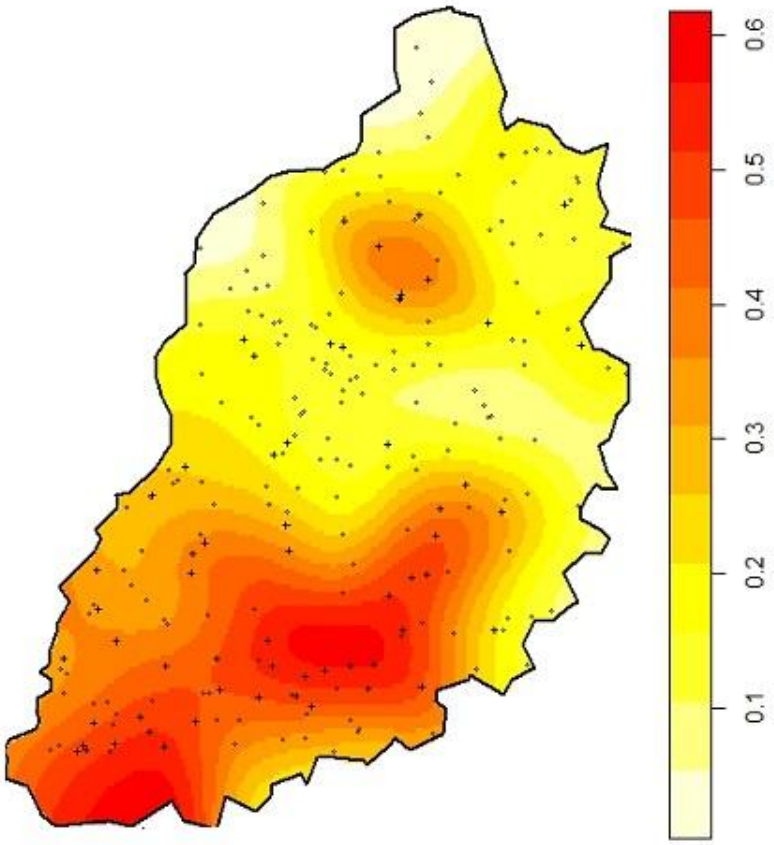


Figure 7.3 Spatially smoothed relative risk for Q fever in cattle

7.4 Discussion

7.4.1 Comparison between slaughterhouse workers and PAZ

a. Knowledge

Slaughterhouse workers are less educated than the PAZ adjusted population (OR 0.3; 95% CI 0.2–0.5 for secondary education). They have the same knowledge of zoonotic disease (OR 1.2; 95% CI 0.9–1.7). Other studies have shown that education is associated with better hygiene practices in slaughterhouses (Ghimire, 2013) and with zoonotic disease awareness (Brown et al., 2011).

b. Disease

Slaughterhouse workers were at increased risk of HIV when compared to the PAZ adjusted population (OR 1.8; 95% CI 1.1–2.9). The increased risk for HIV in this group may be associated with increased risk behaviour, such as consuming alcohol. 32% of slaughterhouse workers reported taking alcohol regularly. Alcohol consumption has been associated with HIV risk in other studies in sub-Saharan Africa. (Fisher et al., 2007). The increased risk of HIV may also be associated with income. Increased wealth has been associated with increase in HIV risk in Kenya (KNBS, 2010).

Slaughterhouse workers were more likely to have malaria (OR 1.5; 95% CI 1.0–2.3) at the time of interview and this may be a factor of their working conditions. Slaughterhouse workers start work before dawn during the risk period for exposure to malaria (WHO, 2014a), and workers perform physical exercise that increases perspiration and carbon dioxide production that attract malaria infected mosquitoes (Smallegange et al., 2013). There may also be standing water around the

slaughterhouse from runoff that might act as mosquito breeding grounds as many slaughterhouses have poor drainage and an open pit system for disposal of waste.

Slaughterhouse workers were more likely to have wounds at interview (OR 2.4; 95% CI 1.3–4.7) which is consistent with other reports that this occupation is a high risk for injury (Burridge et al., 1997, Cai et al., 2005, Pedersen et al., 2010).

c. Self reported symptoms

The univariable logistic regression analysis investigating the difference between slaughterhouse workers and the PAZ adjusted population for a variety of self-reported symptoms indicated that slaughterhouse workers were healthier than the community sample. This finding may suggest selection bias where only active workers were sampled and sick workers were not sampled due to absence on the day of sampling and is generally referred to as the "healthy worker effect" (Li and Sung, 1999). Another element of the "healthy worker effect" is confounding due to non-comparability between the populations since, by the nature of the work, slaughterhouse workers need to be physically strong and able-bodied. In addition, slaughterhouse workers may have more available cash income due to being employed, placing them in a different socioeconomic group and therefore be able to seek health care. This conclusion is supported by the fact that slaughterhouse workers were more likely to have taken medicines in the past 3 months (antibiotic, anti-inflammatory, anti-malarial). Methods for dealing with this selection bias include following up retired or absent workers for inclusion in the study (selection bias) or selecting a more appropriate comparison population (confounding) (Li and Sung, 1999). Efforts were made to reduce confounding by selecting a similarly aged

group from the PAZ dataset for comparison and accounting for age and gender in the univariable models.

d. Seroprevalence of zoonoses

Slaughterhouse workers are potentially more exposed to zoonotic disease than other members of the community in western Kenya. The logistic regression analysis indicates that slaughterhouse workers have 2.3 times the odds of being seropositive for leptospirosis than the PAZ adjusted population and 1.9 times the odds of being seropositive for Q fever. This result, suggesting that slaughterhouse workers are approximately two times more exposed to zoonotic disease, is consistent with other published reports (Chan et al., 1987, Schoonman and Swai, 2009, Sharma et al., 2006).

7.4.2 Spatial risk

a. Leptospirosis

The spatial risk for leptospirosis in the PAZ adjusted population appears to be greatest in the north-eastern corner of the study. These areas are part of Bungoma and Kakamega counties (Figure 2. 1B in Section 2.1) where the main agricultural activity is sugar cane farming. Sugar cane cutting is considered a high risk occupation for leptospirosis exposure (Faine, 1999). This assocation is likely due to rodents inhabiting the sugar cane fields (Emanuel et al., 1964). This connection would need to be quantified further in a more targeted study. There is a medium level risk in the south of the study area bordering Lake Victoria related to fishing, swimming, and contact with infected water as previously discussed. (Jackson et al., 1993, Christova et al., 2003).

The pattern of spatial risk to leptospirosis for the PAZ adjusted population differs from that of slaughterhouse workers. The greatest risk areas for slaughterhouse workers is through the middle of the study area possibly associated with cattle imported from outside the study are for slaughter. Three of the main cattle markets Ogalu, Bumala and Nambale are located in this region (Figure 2.1).

b. Q fever

Q fever in the PAZ human population is clustered in the south of the study area and seems to be associated with areas of the highest cattle density (Figure 7.1). The same applies for Q fever in cattle. Q fever exposure in slaughterhouse workers has a different distribution and is possibly associated with cattle brought in from outside the study area, as described for leptospirosis. It appears from this study that contact with infected animals or living in proximity to infected animals is the risk for exposure to Q fever in this area. This result is similar to those reported in other countries regarding the risk of Q fever (Roest et al., 2011).

7.5 Conclusion

This is the first study in Kenya to compare zoonotic disease risk in slaughterhouse workers to the general population. The hypotheses of the study were:

1. slaughterhouse workers are healthier than the general population
2. slaughterhouse workers are more aware of zoonoses than the general population
3. slaughterhouse workers are more exposed to zoonotic disease than the community
4. the geospatial risk of zoonotic disease in slaughterhouse workers resembles that in the human and cattle population of the study area

In respect to the first hypothesis it can be concluded that slaughterhouse workers are healthier than the general population. They are less likely to report a range of clinical symptoms. However slaughterhouse workers do have increased infectious disease risks. Slaughterhouse workers are at greater risk of malaria infection than the general population which might be related to the hours of the day that they are working. Slaughterhouse workers are more likely to have HIV which may be related to their income.

The second hypothesis is not proven. Slaughterhouse workers are not more informed about zoonoses. Improved education has been linked to zoonotic disease awareness. Slaughterhouse workers are less educated than the general population. Slaughterhouse workers should be more informed about zoonotic disease and zoonotic disease risk. This is not only to protect themselves but also to protect the

meat they handle from becoming contaminated. Slaughterhouse workers need targeted training regarding zoonotic disease risks.

The findings of this study confirm the third hypothesis that slaughterhouse workers are more exposed to zoonotic disease than the general population in western Kenya. This is likely to be related to the number of animals that slaughterhouse workers handle but also a combination of risk behaviours that predispose workers to exposure.

The final hypothesis is not proven. The geospatial risk of zoonotic disease in slaughterhouse workers in western Kenya is not related to the disease risk in people or animals in the study area. The epidemiology of leptospirosis in western Kenya is likely to be complex with a range of circulating serovars from livestock and rodent sources. It does appear that slaughterhouse workers are occupationally exposed to leptospirosis and have a greater risk than the general population. Contact with livestock is a risk factor for the general population as well as other potential sources such as sugar cane farming and contaminated water sources. The geographical range of Q fever within the study areas seems limited to the south. It appears that this is the area of greatest risk to the general population through exposure to infected animals. In contrast, the slaughterhouse workers may exposed to animals being moved from other areas. The epidemiology of Q fever in this area could be studied further.

Chapter 8

Conclusion

This thesis aimed to understand the epidemiology of zoonoses in slaughterhouse workers in western Kenya, not only because it aids our understanding of these diseases but because the workers themselves were concerned about their health and wanted a better understanding of their risks.

The thesis started with 3 hypotheses:

1. Slaughterhouses in western Kenya have inadequate infrastructure, sanitation and hygiene practices
2. The current situation in slaughterhouses in western Kenya contributes to zoonotic disease risk in slaughterhouse workers
3. Slaughterhouse workers are more exposed to zoonotic disease than other members of the community.

The methodology for testing these hypotheses was developed over the preceding chapters:

- A cross-sectional survey of slaughterhouses in the study area was performed to collect data on the current facilities and practices in slaughterhouses
- Slaughterhouse workers were recruited into the study and answered a structured questionnaire on knowledge, practices and risk factors for zoonotic disease
- Biological samples were collected from slaughterhouse workers for the purpose of serological testing for zoonotic and other infections
- Data were analysed using multivariable mixed effects logistic regression models to identify risk factors for exposure to zoonoses

- A community based sample of people of similar age from the region was analysed in the same way

- Comparisons were made between the groups to quantify the difference in risk for zoonotic disease

The first hypothesis stated that the slaughterhouses in the study region lacked infrastructure, sanitation and hygiene. This was explored in Chapter 3. It was found that many slaughterhouses do not have basic infrastructure such as a cement floor or roof. Slaughterhouses are lacking sanitation facilities such as piped water, toilets or hand washing facilities. Antemortem meat inspection is not conducted in the majority of slaughterhouses.

Slaughterhouse workers do not wear protective clothing in the majority of slaughterhouses. Slaughterhouse workers smoke and eat whilst working and some workers appeared intoxicated during their medical examination. Workers were unable to recognise or name zoonotic diseases in animals.

The current conditions in slaughterhouses in western Kenya are far below the recommended standard. In addition, zoonotic disease knowledge and awareness is poor and this perpetuates the substandard conditions in slaughterhouses. There is a lack of impetus or incentive to improve the situation.

The second hypothesis stated that the conditions in slaughterhouses were risk factors for zoonotic disease in workers. This was explored through Chapters 4 and 5 and it was determined that the current conditions in slaughterhouses in western Kenya do pose a risk to slaughterhouse workers. It was shown in Chapter 4 that slaughterhouse

workers have a higher seroprevalence to leptospirosis and Q fever. In addition to this RVF was described in this population for the first time.

In Chapter 5 the risk factors for leptospirosis and Q fever were explored. Slaughterhouse workers at slaughterhouses where antemortem inspection is performed are at decreased risk of leptospirosis as are workers who work in slaughterhouses where protective clothing is worn. Contaminated water supplies at slaughterhouses may also be a source of infection.

Inadequate personal hygiene practices are the most important factors in the risk of exposure to zoonotic disease in slaughterhouses. This is evident in Chapter 5 in which risks of leptospirosis seropositivity include smoking and eating at work and having wounds. Risks of Q fever seropositivity include being intoxicated at work.

The third hypothesis was that slaughterhouse workers are more exposed to zoonoses than people in the community. This was tested in Chapter 7. This study was able to show that slaughterhouse workers have twice the odds of being seropositive for leptospirosis and Q fever than the general population. It can be concluded that slaughterhouse workers are at greater risk of exposure to zoonotic disease than people in the community. Interestingly this risk is not geospatially related to disease in the human population. This implies that the transmission of zoonotic disease to slaughterhouse workers may be from animals being imported to the region for slaughter.

Recommendations

The most important outcome of this is study is to report these findings to the county veterinary department in Kenya's newly devolved government and to the workers so

that they are informed of their disease risks and how to prevent them. Broadly, recommendations will focus on three areas: facilities/sanitation, knowledge and regulations.

Facilities/Sanitation

Ideally attention should be focused on bigger slaughterhouses where the costs of improved sanitation are not prohibitive. However, until there are improved facilities for the transportation of refrigerated meat this is not a realistic option in western Kenya. Changes to the distribution of meat in western Kenya are also likely to increase the cost of meat to the consumer. This may increase the rate of informal or back yard slaughter. The most important change initially would be the provision of potable piped water for hand washing and cleaning the slaughterhouse adequately with soap and water.

The importance of antemortem inspection should be emphasised to the slaughterhouse and the veterinary department. This may require the training of more meat inspectors in the region. The workers also play an important role in recognising disease in animals and this leads to the next area for intervention.

Knowledge

Slaughterhouse workers need to be aware of the risks to their health from zoonotic diseases. This requires education about the disease, how to recognise clinical signs in animals and how to prevent transmission. Diseased animals will enter the slaughterhouse because producers sell sick animals to recoup losses. Slaughterhouse workers need to know how to reduce their risks of exposure to disease from these

animals. Combined with this training would be education about producing a clean and safe meat product.

Education should not be limited to slaughterhouse workers. The general public should be more aware of the risks of eating contaminated or infected meat and encouraged to demand a better product. This will put pressure on butchers to improve facilities for slaughter.

The veterinary department also needs to be aware of the diseases that are of importance to veterinary public health in the region through reporting which should form part of the regulations.

Regulations and reporting

The Department of Veterinary Services is responsible for the training and provision of meat inspectors. Meat inspectors need to be adequately trained in the importance of antemortem and postmortem inspection but there also needs to be enough meat inspectors to conduct meat inspection across the region. Unfortunately the distances between slaughterhouses combined with poor roads and insufficient transport make it difficult for meat inspectors to reach all their assigned facilities in time.

There need to be adequate reporting structures to follow cases of disease back to the farm. The poverty in the region means that there is a disincentive to cull animals for disease. Hence cases of disease in animals are unlikely to be reported. Producers need to be educated to the benefits of improved herd health by removing diseased animals. This is the role of the veterinary department.

Limitations

One of the factors against improving conditions in slaughterhouses is the cost. As mentioned previously it is the local butchers who coordinate the slaughtering process in western Kenya. The average return for a beef butcher in Kenya is US$50 per animal. The smaller slaughterhouses in western Kenya are slaughtering less than one animal per day. There is little incentive to improve conditions or invest in improved hygiene when the costs will diminish the financial returns to the butcher. The government charges for meat inspection are US$2 for cattle, US$0.80 for goats and US$0.30 for pigs. For meat inspectors in western Kenya there is little incentive to inspect meat at the smaller pig slaughterhouses as the cost of transport is likely to be greater than the fee. Slaughterhouse workers are paid US$1.10 per animal slaughtered in cattle slaughterhouses. Workers do not have the financial means to purchase protective clothing, seek medical attention when required or abstain from work if they are sick.

Raising awareness

“Prevention is better than cure” is an ancient idiom that we all know, particularly when it comes to our own health and well-being. Unfortunately in the developing world the systems that should predict and prevent public health and veterinary public health disasters are weakened by underfunding, inadequate expertise and insufficient incentive.

The answer is not simply more expert scientists to further our understanding of zoonotic diseases and their pathogenesis. A greater understanding of the context of the situation in Africa is needed including the social determinants of disease. An

adequate public health and veterinary public health infrastructure is required to support the rolling out of control measures that include education, surveillance, quarantine, reporting and an appropriate response.

There is a drive to invest in technology to combat disease in Africa, to improve diagnostics and develop vaccines. There is investment in emerging disease detection given the current concerns about a global pandemic. There is unfortunately not the same push to develop local infrastructure to support surveillance, reporting and control of endemic disease. We still do not know the burden of brucellosis and bovine tuberculosis in the developing world despite most industrialised nations being able to control and even eradicate these diseases in their own countries.

A deeper understanding of the epidemiology of these diseases is needed through primary research, and alongside this a better understanding of the social epidemiology and drivers of disease. However without adequate means to use these data and respond appropriately this information is useless to policy makers in developing countries.

In order to implement changes in the slaughterhouses in western Kenya there needs to be education of the workers, the butchers and the slaughterhouse owners of the risks. There need to be incentives or disincentives to change conditions to protect worker health. The risks related to poor hygiene in slaughterhouses are not just worker health but also to the community. Contaminated meat can lead to the spread of bacterial pathogens such as *E. coli* to the community. Education of the community is required to buy meat that is produced in a safe manner and hence put pressure on butchers to improve conditions.

Rapid enforcement of changes in this region is likely to result in an increase in meat coming from the informal market or home slaughter. A balance is required that protects the worker and the consumer from disease. It is this authors considered opinion that training and education should be at the forefront of any interventions regarding zoonotic disease prevention in western Kenya.

APPENDICES

Appendix 1 Consent form

Study title: Slaughterhouse workers as sentinels of zoonotic disease emergence

Instructions

- **Enumerator to distribute read and explain to participant. Use English, Swahili or local language as appropriate**
- **One signed copy for hardcopy file, one signed copy for participant**

We are visiting you to invite you to participate in a research project which aims to understand the importance of zoonotic diseases in your community. Zoonotic diseases are diseases that you may get from direct or indirect contact with animals. Our objective is ultimately to learn to control these diseases better, and in particular, understand how controlling such diseases may prevent them from infecting people. This is a research project run jointly by the Kenya Medical Research Institute (KEMRI), International Livestock Research Institute (ILRI) in Nairobi and the University of Edinburgh (UK). It is funded by the Wellcome Trust in the UK.

To carry out this research, we would like to ask you some questions about your work, the animals you work with, your health and health problems and also collect some samples for further detailed analysis. The outcome of this research will be a better understanding of zoonotic diseases. Findings from this investigation will help us advise both human and animal health authorities in your region and the rest of Kenya and beyond about improving health.

What is involved

Your participation will take approximately 30 minutes of your time. You have been selected for this project because you work in a slaughterhouse. You are free to decline if you would prefer not to take part. Taking part will involve:

1) Answering some general questions about your health and your work
2) Allowing us to take measurements such as your height and weight
3) Providing us with a sample of your faeces to look for parasites like worms
4) Providing is with a sample of your urine
5) Providing us with a sample of your sputum
6) Allowing our qualified technician/nurse to take a 15ml blood sample from your arm - equivalent of 1 tablespoon. So that we can take these samples to the laboratory (in Busia) and examine the blood for infections that you may have or have had in the past and that can be detected in your blood.
7) Allowing our qualified technician/nurse to take a nasal swab.

Measurements and samples will be taken by a qualified clinician or technician. There will be some discomfort associated with sampling blood, which will use a needle to collect blood from your arm. This discomfort is transient.

Benefits to participants

We will offer you a general health check as part of this study – by taking measurements like your height and weight and conducting an examination, we can advise you if you appear in good or bad health and suggest whether we think you should attend a clinic for further tests. We will advise you of the most appropriate facility for further consultation if required. If you would like us to, we can also prepare a report which we will send to you to inform you of what parasites we find in your faecal sample and blood sample – eg worms, malaria. This health check and parasitology report that we are offering is free of charge to you, and if you

choose not to participate in the sampling of the project, we will none-the-less carry out the health check if you wish: participation is thus entirely voluntary and there is no consequence to you for not participating should you choose not to.

Anonymity/secondary use of material

Beyond the health check and parasitology tests, your participation will be totally anonymous. We will conduct further tests for a range of diseases on your sample, but it will no longer be possible for us to identify you individually with your test results – the link between your identity and your test results can therefore not be shared with anyone, and your name will never appear in any reports. These anonymous samples will be stored and analysed at ILRI or KEMRI or an appropriate international laboratory, and, while remaining anonymous, may be used for further work on them in the future. Afterwards, samples will be stored and there may be further examination of your samples, but again, these analyses will be anonymous and cannot be linked to you individually. As we will be unable to locate a specific individual's samples from our storage (because the storage is anonymous), agreement to participate makes implicit your agreement for the materials to be used in future studies. Your answers to our questions, measurements and results will remain completely confidential to all involved at all stages of the project – even other members of the project team will not be able to link specific samples to you or even to know the name of the village the samples came from. In the case of the parasitology results, if you choose to receive the results of these tests, we will indicate on the report what steps you might follow for medical follow-up – eg visiting your local district hospital.

The project has been reviewed and approved by KEMRI/Kenya National Ethical Review Committee. For further questions, please contact Dr. Eric Fèvre, Busia PAZ/IDEAL Laboratory, PO BOX 261, Busia for detailed questions or worries after the team has left (tel Busia 05522233), or the Secretary, KEMRI Ethical Review Committee (020 272 2541) if you have any concerns.

Participant statement

I confirm that I have understood the above description of the study and that I have had the opportunity to ask questions about this study that I wish to ask. I confirm that I am happy to provide answers to the questions that will be asked of me and that I am happy to allow the project team to take the necessary samples for this project. I confirm that my samples may be stored and shipped as is necessary for the completion of this project and may be stored beyond the project for further medical research. I am aware that from the point of collection, I will not be personally identifiable; I understand that the project will not routinely report back the specific results of the tests to be carried out on my samples.

_____	_____
Date	**Sublocation name**
_____	_____
Participant name	**Signature or thumb print**

Enumerator statement

I confirm that I have fully explained to the subject the nature and purpose of the procedures described above. Explained any risks and described the system of anonymous data gathering. I have asked the subject if he or she has any further questions, and answered these questions to the best of my ability.

Name	**Signature**

Appendix 2 Slaughterhouse individual questionnaire

General

1. Date --/--/----
2. Start time --:--
3. Recorder <name> (look up list)
   Annie, Cheryl, Daniel, Fred, Isaac, James, Lauren,Maseno, Oliver, Omoto
4. Slaughterhouse barcode <number> Scan
5. Respondent age: -- (in age groups – as people do not know exact age)
6. Respondent sex: Male / Female
    a. If female are you pregnant? Y/N/NR
        i. If pregnant What stage? 1st, 2nd, 3rd trimester
7. Does this participant meet the selection requirement (over 18) and given informed consent? Yes / No (Terminate)
8. Interviewee barcode SCAN <number>
9. Language of questionnaire administration (Language look up)
   Teso; Samia; Bukusu; Luhya; Luo; Swahili; English; Kamba; Kalenjin; Other
10. Tribal origin (Tribe look up)
    Teso; Luhya; Luo; Kikuyu; Samia; Saboat; European; Kamba; Kalenjin; Other
11. Principal religion (religion look up)
    Roman catholic; Protestant; Other Christian; Muslim; Traditional religion; None; Other; NR
12. Marital status (marital look up)
    Single; Married; Divorced; Widowed; NR
13. Do you have children? Y/N/NR
    a. If yes, how many living? (popup table – numbers up to 30)
14. Have you lived outside this province at any time for more than 6 months? Y/N/NR
    a. Where did you live? Popup list provinces
15. How many people live in your homestead? (popup list numbers)
16. How many rooms in your homestead? (popup list numbers)
17. What level of education have you reached? (look up education)
    No formal education; Pre-school; Primary; Secondary; Tertiary; College; University; Vocational/technical school; Other; NR
18. Do any members of your homestead work in healthcare? Y/N/NR
    a. What do they do? (look up healthcare)
    Doctor, nurse, midwife, clinical officer, nursing assistant, admin, cleaner, security, NR. other
19. Do you have contact with livestock outside of work? Y/N/NR
    a. Which ones? (multiselection list)
    Cattle, sheep, goats, pigs, poultry, rabbits, NR, Other
20. Do you or your family keep livestock at you normal place of residence? Y/N/NR
    a. Which ones? (multiselection list)
    Cattle, sheep, goats, pigs, poultry, rabbits, NR, Other
21. Do any members of your homestead work in livestock farming? Y/N/NR
    a. What animals?
    Cattle, sheep, goats, pigs, poultry, rabbits, NR, Other
22. Do you have contact with dogs? (look up freq)

No; Daily; At least once a week; At least once per month; At least once per year; Used to but no longer; NR

23. In the last 12 months have you been hunting? (look up freq)
    No; Daily; At least once a week; At least once per month; At least once per year; Used to but no longer; NR

Food preferences

24. Do you eat beef? Y/N/NR
    a. How often do you eat beef? (look up freq)
    Never; Daily; At least once a week; At least once per month; At least once per year; Only on special occasions; Used to but no longer; NR
25. Do you eat pork? Y/N/NR
    a. How often do you eat pork? (look up freq)
    Never; Daily; At least once a week; At least once per month; At least once per year; Only on special occasions; Used to but no longer; ND
26. In the last 12m have you drunk cow's milk? Yes No NR
    a. How do you take your milk? (multiselection)
    Boiled; Soured; Raw; Pasteurised; Other (allow all answers)
27. In the last 12m have you drunk goat's milk? Yes No NR
    a. How do you take your milk? (multiselection)
    Boiled; Soured; Raw; Pasteurised; Other
28. Do you take animal blood? Yes No NR
    a. How do you take animal blood? (multiselection)
    Boiled, Cooked, Raw, Other
29. Do you smoke cigarettes? (look up table freq)
    No; Daily; At least once a week; At least once per month; At least once per year; Used to but no longer; NR
    a. If daily or weekly what is the number of cigarettes you smoke per week? <number>
30. Do you consume alcohol? (look up freq)
    No; Daily; At least once a week; At least once per month; At least once per year; Used to but no longer; NR

Sanitation

31. Where do you obtain water (for personal use)? (look up water)
    Private borehole; River; Shared borehole; Municipal water (tap); Well; Spring; Dam; Pond: Other; Lake; NR
32. How often do you use the latrine when you need to defecate? (look up defacation)
    Everytime; Mostly; Sometimes; Rarely; Never; NR

Health status

33. Have you had a period of illness in the past 12 months?
    Yes No NR
    a. If you have had a health problem, please list <open>
34. Have you had fever in the last 3 months? Y/N/NR
35. Have you had headache in the last 3 months? Y/N/NR
36. Have you had backache in the last 3 months? Y/N/NR
37. Have you had joint pain in the last 3 months? Y/N/NR
38. Have you had seizures in the last 3 months? Y/N/NR

39. Have you had weightloss in the last 3 months? Y/N/NR
40. Have you had cough in the last 3 months? Y/N/NR
41. Have you had nausea in the last 3 months? Y/N/NR
42. Have you had vomiting in the last 3 months? Y/N/NR
43. Have you had diarrhoea in the last 3 months? Y/N/NR
44. Have you had loss of appetite in the last 3 months? Y/N/NR
45. Have you had abdominal pain in the last 3 months? Y/N/NR
46. Have you had a skin infection in the last 3 months? Y/N/NR
47. Have you had any boil in the last 3 months? Y/N/NR
48. Have you taken any medicines in the last 3months
    Yes No Don't know NR
    a. If you have taken medications in the last month, tick all that apply
    Unknown; Chloroquine; Other antemalarial; Anti-retroviral; Antibiotic; Anti-tussive; Rehydration solution; Antiinflammatory or pain killer; Heart medication; Insulin; Medication for seizures; Dewormers; Other; ND
49. Have you used any antimicrobial cream or ointment in the last 3 months? Y/N/NR
50. Usually, when you feel ill, where do you seek treatment? (look up treatment)
    Don't seek treatment; Family member; Community health worker; Traditional healer; Chemist; Private clinic; Hospital; Self treatment; Neighbour; Church healer; Other; NR
51. Have you visited a clinic, community health centre or doctor in the last 3 months? Y/N/NR
52. Have you visited a hospital in the last 3 months? Y/N/NR
    a. Have you been admitted (stayed overnight) to a hospital in the last 3months? Y/N/NR
53. Are you aware of ever having brucellosis?
    Yes No Don't know NR
54. Are you aware of ever having tuberculosis?
    Yes No Don't know NR
55. Are you aware of ever having q fever?
    Yes No Don't know NR
56. Are you aware of ever having tapeworm?
    Yes No Don't know NR
57. Are you aware of ever having Rift Valley fever?
    Yes No Don't know NR

Knowledge of food-borne and zoonotic disease

58. Are you aware of any disease you might catch from contact with animals?
    Yes No Don't know NR
    a. Which symptoms/disease might you catch from animals? (multiselection)
    Unknown disease; Malaria; Fever; Stomach pain; Diarrhoea; Respiratory difficulties; Fever; Skin rash; Seizures; Brucellosis; Anthrax; TB; RVF; Q fever; Sleeping sickness; Tapeworm; Leptospirosis; Salmonella; E.coli; Rabies; Toxoplasma; Other; NR
    If answered other please type_____
59. Are you aware of any diseases that you might catch from eating meat? Y N DK NR
    a. Which symptoms/disease might you catch from eating meat (multiselection)
    Unknown disease; Malaria; Fever; Stomach pain; Diarrhoea; Respiratory difficulties; Fever; Skin rash; Seizures; Brucellosis; Anthrax; TB; RVF; Q fever;

Sleeping sickness; Tapeworm; Leptospirosis; Salmonella; E.coli; Rabies; Toxoplasma; Other; NR

If answered other please type_____

Slaughterhouse questions

60. How long have you been a slaughterhouse worker? <number years>
61. How long have you worked in this slaughterhouse? <number years>
62. Do you work in another slaughterhouse at present? Yes No NR
63. Which other SH? _____
64. How many days per week do you work as a slaughterhouse worker? 1 2 3 4 5 6 7
65. How many hours per day do you work? 1 2 3 4 5 6 7 8 9 10 11 12
66. What is your occupation in the SH? (look up Job in SH)
    Slaughterman; Flayer; SH owner; Butchery owner; Clean the intestines; Cleaner; Other: NR
67. Do you have another occupation? Yes /No/NR
68. What is this occupation? (popup Y/N/NR)
    a. What is this occupation? (look up occupation)

Meat business owner; Farmer; Trader; Shop keeper; Student; Driver; Butcher; Fisherman; Boda driver; Carpenter; Mason; Taxi driver; Other; NR

If other enter details:

69. Are you involved directly in slaughter/flaying? Y/N/NR
    a. How many animals do you personally slaughter a day on average? <number>
    b. Who provides you equipment? Look up list
       Butcher; Meat inspector; SH owner; Worker; Other; NR
    c. Is your equipment used exclusively within the slaughterhouse? Y N DK
    d. How often do you clean your equipment? (look up wash freq)
    Between animals, Before slaughtering; After slaughtering; Daily; Weekly; Never; NR
    e. What do you use to clean your equipment? (multilist cleaning)
    Water; Bleach; Ammonia; Soap; Washing powder; Nothing; NR
    f. How often do you or someone else sharpen your equipment? (look up freq)
    Daily; Weekly; Monthly; Never; NR
    g. Have you been for a medical check up recently (in the last 6 months)? Y/N/NR
    h. Do you have a license for slaughtering? Y/N/NR
70. Do you wear protective clothing eg. Coveralls, overalls, apron? Always/Sometimes/Never
    a. What protective clothing? (Look up clothing)
       Coveralls, Overalls, Apron, Lab coat, Other. NR
    b. Who provides?
       Meat inspector; Butcher; Worker; SH owner; Other; NR
71. Do you change clothes when you leave the slaughterhouse?
    Yes No NR
72. Where do you wash your clothes/shoes after slaughtering?
    River; Home; At the slab; Give to someone; Other; NR
73. Do you wear footwear in the slaughterhouse? Y/N/NR Always/Sometimes/Never
    a. What type?
       Sandals, Boots, Shoes, Rubber boots, Running shoes, Other, NR

b. Who provides?

Meat inspector; Butcher; Worker; SH owner; Other; NR

74. Do you wear gloves when slaughtering? Always/Sometimes/Never
75. When do you wash your hands? (tick all that apply) (multiselection)
    Before slaughtering; After slaughtering; Between animals; Before I go home; After I use the latrine; NR
76. Is there soap provided for hand washing?
    Always/Sometimes/Never
77. Do you injure yourself at work and how often? (look up freq)
    Daily, Weekly, Monthly, Never
78. Do you eat at the slaughterhouse? (Look up meat inspector freq)
    Every time we slaughter; Most times(once a week); Sometimes (once a month); Rarely (once a year); Never; NR; DK
79. What would you do with a sick animal? (look up sick animal)
    Send home; slaughter last and condemn; slaughter and sell; slaughter and keep for own consumption, Treat, Ask doctor, Slaughter and ask doctor, Other, NR
80. What would you do with an animal that dies on the way or at the slaughterhouse?
    Send home; slaughter last and condemn; slaughter and sell; slaughter and keep for own consumption, Treat, Ask doctor, Slaughter and ask doctor, Other, NR
81. What animals do you slaughter/flay/clean?
    Cattle only, Sheep/goats only; Cattle and Sheep/goats; Pig only; Pigs and cattle; Pigs and sheep/goats; NR

    IF cattle

82. Have you seen these lesions (show picture of tuberculous lung/liver)? Pictures are available in appendix 7

    Yes; No; Don't know; NR

    If yes:

    a. How often? (look up freq)
       Daily; Weekly; Monthly; Yearly; DK
    b. Named correctly Yes; No;
    c. What do you do with these animals? (look up organ disposal)
       Slaughter as normal, Dispose of entire carcass, Remove affected organs

83. Have you seen these lesions (show picture of brucellosis lesions)?
    Yes; No; Don't know; NR
    If yes:

    a. How often? (look up freq)
       Daily; Weekly; Monthly; Yearly; DK
    b. Named correctly Yes; No;
    c. What do you do with these animals? (look up organ disposal)
       Slaughter as normal, Dispose of, Remove affected organs

84. Have you seen these lesions (show picture of skin lesions on people)?
    Yes; No; Don't know; NR
    If yes:

    a. How often? (look up freq)
       Daily; Weekly; Monthly; Yearly; DK
    b. Named correctly Yes; No;

85. Have you seen these lesions (show picture of echinococcus)?
    Yes; No; Don't know; NR
    If yes:

    a. How often? (look up freq)

Daily; Weekly; Monthly; Yearly; DK

b. Named correctly Yes; No;

c. What do you do with these animals? (look up organ disposal)
Slaughter as normal, Dispose of, Remove affected organs

If pigs

86. Have you seen these lesions (show picture of *Taenia* cysts)?
Yes; No; Don't know; NR
If yes:
a. How often? (look up freq)
Daily; Weekly; Monthly; Yearly; DK
b. Named correctly Yes; No;
c. What do you do with these animals? (look up organ disposal)
Slaughter as normal, Dispose of, Remove affected organs

87. How often does the meat inspector visit? (look up freq)
Every time we slaughter; Most times (once a week); Sometimes (once a month); Rarely (once a year); Never; NR; DK

88. Does the meat inspector examine the animals before they are slaughtered?
Always, Sometimes, Never, NR
a. Does he/she ever refuse the slaughtering of an animal?
Yes/No/NR
b. How often does the meat inspector refuse to allow an animal to be slaughtered?
Every time we slaughter; Most times (once a week); Sometimes (once a month); Rarely (once a year); Never; NR; DK
c. For what reason would the meat inspector refuse slaughter? Multilist
Sickness, Diarrhoea, Coughing, Injury, Emaciation, Death. Other. DK, NR

89. How often does the meat inspector ever condemn animal or part of an animal (organ)? (look up freq)
Every time we slaughter; Most times (once a week); Sometimes (once a month); Rarely (once a year); Never; NR; DK
a. Which parts? Liver, Kidney, Heart. Lung, Intestines, Muscle
b. What happens to these organs? (look up organ disposal)
Pit, Dog, Home

90. Does the meat inspector ever condemn an entire carcass? Y/N/NR
a. How often?
Every time we slaughter; Most times (once a week); Sometimes (once a month); Rarely (once a year); Never; NR; DK
b. What happens to the carcass?
Pit, Dog, Home

91. In the last 12 months have you seen rats around the slaughterhouse? (look up freq)
No; Daily; At least once a week; At least once per month; At least once per year; Used to but no longer; NR

92. In the last 12 months have you seen wildlife around the slaughterhouse? (look up freq)
No; Daily; At least once a week; At least once per month; At least once per year; Used to but no longer; NR
a. What wildlife have you seen? (multiselection list)

Bats, rodents, Mongoose, birds, Snakes, lizards, monkeys, other, NR

Observational

93. Weight
94. Height
95. Midupper Arm Circumference
96. Temperature
97. Splenomegaly
98. Hepatomegaly
99. Abdominal distension
100. Membrane palour
101. Jaundice
102. Limb oedema
103. Rash
104. Wounds requiring treatment
105. Does the individual have a BCG scar?
106. Does the worker appear drunk?
107. Red top barcode
108. Purple top bar code
109. Was a stool sample collected?
    a. Barcode
110. Was a stool swab sample collected?
    a. barcode
111. Was a sputum sample collected?
    a. barcode
112. Was a nasal swab sample collected?
    a. barcode
113. Thick film barcode
114. Thin film barcode

Appendix 3 Slaughterhouse foremen questionnaire

1. Date --/--/----
2. Start time --:--
3. Recorder <name>
4. Slaughterhouse barcode <number>
5. Sex of respondent
6. Language of questionnaire
7. Job in slaughterhouse
8. How many slaughter men
9. How many are flayers
10. How many are licensed
11. Which animals are slaughtered?
    Cattle only, Cattle and sheep/goats, Pigs only, Pigs and cattle, Pigs and sheep/goats.
    If cattle/sheep/goats
    Number of cattle slaughtered per week (average)
    How are the cattle transported?

    Number of sheep/goats slaughtered per week (average)
    How are the sheep/goats transported?

    If pigs
    Number of pigs slaughtered per week (average)
    How are the pigs transported?

12. Where is the meat from this slaughterhouse sold?
    Locally
    Exported to other districts
13. How is the meat transported?
14. What would you do with a sick animal?
15. What would you do with a dead animal?

16. How often does the meat inspector visit the slaughterhouse?
        Daily Weekly Fortnightly Monthly

17. Does the meat inspector examine animals before slaughter?
18. Does the meat inspector refuse to allow slaughter?
19. For what reasons does he refuse to allow slaughter?
20. How often?
21. Does the meat inspector condemn organs?
22. How often does the meat inspector condemn organs?

23. For what reason does the meat inspector condemn organs?

24. What organs does the meat inspector most commonly condemn?

25. Does the meat inspector condemn the entire carcass?
26. How often does the meat inspector condemn the carcass?

27. For what reason does the meat inspector condemn the carcass?

28. What method of euthanasia do you employ?

29. Is specialized protective clothing worn whilst in the slaughterhouse?
    Y N DK
30. Type: Overalls, Apron, Lab coat
31. Who provides this clothing?
32. Is this clothing worn exclusively in the slaughterhouse?
33. Where is this clothing/footwear cleaned/laundered?
    At the slab, at individual homes,
34. Is specialized protective footwear worn whilst in the slaughterhouse?
    Y N DK
35. Type Boots, Closed shoes, Slippers
36. Is this provided by the SH? Y N DK
37. What specialized equipment is used within the slaughterhouse?
    Winch; Trolley; Saw; knives; Axe
38. How often is the equipment cleaned?
    Between animals, Before slaughtering; After slaughtering; Daily; Weekly; Never; NR
39. What do you use to clean your equipment?
    Water; Bleach; Ammonia; Soap; Nothing; NR
40. Is this equipment used exclusively within the slaughterhouse? Y N DK
41. How often do you clean the slab?
    Between animals, Before slaughtering; After slaughtering; Daily; Weekly; Never; NR
42. What do you use to clean the slab?
    Water; Bleach; Ammonia; Soap; Nothing; NR
43. Do dogs come to the slaughterhouse?
    Never; Daily; At least once a week; At least once per month; At least once per year; Used to but no longer; ND
    a. Do you feed the dogs internal organs of animals?
       Always Sometimes Never
44. Do cats come to the slaughterhouse?
    Never; Daily; At least once a week; At least once per month; At least once per year; Used to but no longer; ND
    a. Do you feed the cats internal organs of animals?
       Always Sometimes Never
45. What disposal method is employed for carcass waste?
    Pit; Bury; Throw away; Feed to dogs; Sell; Take home; NR
46. What disposal method is employed for condemned carcasses?
    Pit; Bury; Throw away; Feed to dogs; Sell; Take home; NR
47. Where do you obtain your water for slaughtering?
    Borehole; River; Pump; Tap; Well; Spring; Other; NR
48. Is there a place for hand washing? Y/N
49. Is soap provided for hand washing? Y/N
50. Is there a latrine? Y/N
51. In the last 12 months have you seen rats around the slaughterhouse?
    No; Daily; At least once a week; At least once per month; At least once per year; Used to but no longer; ND
52. In the last 12 months have you seen wildlife around the slaughterhouse?

No; Daily; At least once a week; At least once per month; At least once per year; Used to but no longer; ND

53. What wildlife have you seen around the slaughterhouse?
    Bushbuck; Other bovid; Bats; Mongoose; Snake; Monitor lizard; Monkey; Wild birds; Other

54. Structure of slaughterhouse floor
    Earth Cement Tile Timber
55. Structure of sides
    No sides Timber Unburnt bricks Burnt bricks Stone Cement Mud
56. Structure of roof
    No roof Tile Thatch Iron sheets
57. Is there electricity in the slaughterhouse? Y/N
58. How is water access?
59. Is there a latrine in the compound?
60. Type of latrine
    Latrine completely closed Partially closed Open pit
61. Evidence of latrine use
62. Evidence of animal scavenging around the latrine
63. Did you see workers wearing PPE>
64. Type?
65. Did you see workers wearing shoes?
66. Type
67. Did you see workers wearing gloves?
68. Name of market
69. GPS Northing
70. GPS Easting
71. Altitude
72. GPS code

Appendix 4 Details of closed and declined slaughterhouses

Variable	Declined to participate		Closed during 2011	
	Cattle n=4	Pig n=10	Cattle n=12	Pig n=12
Roof - iron	1	0	5	3
Floor -cement	3	5	12	8
Walls - bricks	0	2	5	6
Electricity	0	0	0	0
Toilet	1	3	9	6
Piped water	0	0	0	0
Hand washing	0	0	0	0

Table A1: Details regarding the slaughterhouses that declined to participate in the study and slaughterhouses that were closed between 2011 and 2012

Appendix 5 Results from cross sectional survey

Table A2: Infrastructure and practices of the slaughterhouses as reported by foreman

Variable	Mixed Ruminant% n=31^	Cattle % (95%CI) n=53	Pigs only % (95%CI) n=58	Total % (95%CI) n=142	Chi squared, p –value
Structural factors					
Roof present	90	75 (72-79)	45 (40-50)	65 (63-67)	X^2=21.53, df=2 p<0.001
Cement floor	100	100	74 (70-78)	89 (87-90)	X^2=23.39, df=2 p<0.001
Solid walls	97	79 (76-82)	53 (48-58)	72 (69-74)	X^2=20.25, df=2 p<0.001
Electricity	3	0	2 (0.4-3)	1.4 (1-2)	X^2=1.48, df=2 p<0.001
Sanitation					
Toilet	61	57 (53-60)	62 (57-67)	60 (57-62)	X^2=0.38, df=2 p=0.117
Piped water[ł]	6	6 (4-7)	0	3 (3-4)	X^2=3.82, df=2 p<0.001
Source of water Borehole Municipal River Well	 65 13 3 19	 70 (67-73) 13 (11-16) 8 (6-9) 9 (7-12)	 64 (59-69) 7 (4-9) 10 (7-13) 19 (15-23)	 66 (64-68) 10 (9-12) 8 (7-9) 16 (14-17)	X^2=3.83, df=2 p<0.001
Hand-washing place	35	19 (16-22)	14 (10-17)	20 (18-22)	X^2=5.76, df=2 p=<0.001
Cleaned with soap	90	83 (80-86)	62 (57-67)	75 (73-78)	X^2=10.92, df=2 p<0.001
Dogs present	71	74 (70-78)	85 (81-88)	78 (76-80)	X^2=2.89, df=2 p<0.001
Rats present	10	6 (4-7)	19 (15-23)	12 (11-14)	X^2=4.87, df=2 p<0.001
Personal hygiene					
Protective clothing worn	55	36 (32-39)	17 (13-21)	32 (29-34)	X^2=13.38, df=2 p<0.001
Worker buys clothing	90 (87-93)	78 (71-85)	67 (51-82)	78 (73-84)	
Boots worn	52	45 (41-48)	16 (12-19)	34 (31-36)	X^2=16.33, df=2 p<0.001
Worker buys boots	92 (87-97)	84 (77-91)	72 (54-90)	84 (78-89	
Soap provided	81	64 (61-68)	57 (52-62)	64 (62-67)	X^2=4.75, df=2 p<0.001
Meat inspection					

Meat inspector visits daily	100	100	84 (81-88)	93 (92-95)	X^2=13.36, df=2 p<0.001
Antemortem exam	13	6 (4-7)	5 (3-7)	7 (6-8)	X^2=1.99, df=2 p<0.001
Slaughter a sick animal	19	8 (6-9)	5 (3-7)	9 (7-10)	X^2=3.69, df=2 p<0.001
Meat exported					
Meat sold only to local village	10	30 (27-33)	29 (25-34)	26 (24-28)	
Meat exported from sublocation	26	19 (16-22)	19 (15-23)	20 (18-22)	

Table A3: Structure and practices of the slaughterhouses as observed by the interviewer

	Mixed % (95%CI) n=28	**Cattle % (95%CI) n=31**	**Pigs only % (95%CI) n=25**	**Total % (95%CI) n=84**
Sanitation				
Pit	100	100	84 (72-96)	93 (89-97)
Toilet	57 (51-63)	65 (53-76)	56 (40-72)	60 (52-67)
Hand washing place	32 (27-38)	10 (3-17)	4 (2-10)	12 (7-16)
Dogs present	64 (59-70)	97(93-100)	80 (67-93)	83 (77-89)
Personal hygiene				
Protective clothing worn >50% workers	64(59-70)	35(24-47)	4(0-10)	27(21-34)
Boots worn by >50% workers	57(51-63)	26(15-36)	4(0-10)	22(17-28)
Soap provided	50 (44-56)	16 (7-25)	12 (2-22)	21 (15-27)
Eating observed	18 (13-22)	6 (1-12)	28 (14-42)	18 (12-24)
Meat inspection				
Meat inspector seen	79 (74-83)	65 (53-76)	32 (17-47)	53 (45-61)
Antemortem inspection	14(10-18)	10(3-17)	0	6(3-10)

Table A4: Demographics of slaughterhouse workers in western Kenya

Variable	Mixed % (95%CI) n=274	Cattle % (95%CI) n=292	Pigs only % (95%CI) n=172	Total % (95%CI) n=738	Chi squared
Gender					
Male	96(94-97)	97(97-98)	97(96-98)	97(96-97)	X^2=0.96, df=2 p=0.188
Female	4(3-6)	3(2-4)	3(2-4)	3(3-4)	
Age group (years)					
18-27	20(17-23)	19(17-21)	37(33-41)	23(21-25)	X^2=51.60, df=6 p<0.001
28-37	31(27-34)	27(25-30)	35(31-39)	30(28-32)	
38-47	19(16-22)	21(19-24)	22(18-25)	20(19-22)	
49+	31(27-34)	32(30-35)	6(4-7)	26(24-28)	
Education					
None	10(8-12)	14(12-16)	6(4-7)	10(9-12)	X^2=8.97, df=4 p<0.001
Primary	75(71-78)	70(68-73)	81(78-84)	74(73-76)	
Secondary	16(13-18)	16(14-18)	13(10-16)	15(14-17)	
Duration of work (years)					
<5	38(35-42)	50(47-53)	56(51-60)	46(44-48)	X^2=39.19, df=6 p<0.001
6-10	26(23-30)	22(20-25)	30(26-34)	26(24-27)	
11-15	15(12-18)	6(5-8)	9(7-12)	11(9-12)	
16+	20(17-23)	21(19-24)	5(3-7)	17(16-19)	
Job in the slaughterhouse					
Cleaner	4(3-5)	5(4-6)	5(3-7)	4(4-5)	
Cleans intestines	8(6-10)	7(6-9)	0	6(5-7)	
Flayer	75(72-78)	74(72-77)	93(91-95)	71(69-73)	
Slaughterman	11(9-14)	12(10-14)	0*	9(8-10)	
Other	2(1-3)	1(1-2)	2(1-3)	2(1-2)	

Table A5: Personal hygiene and sanitation in slaughterhouses

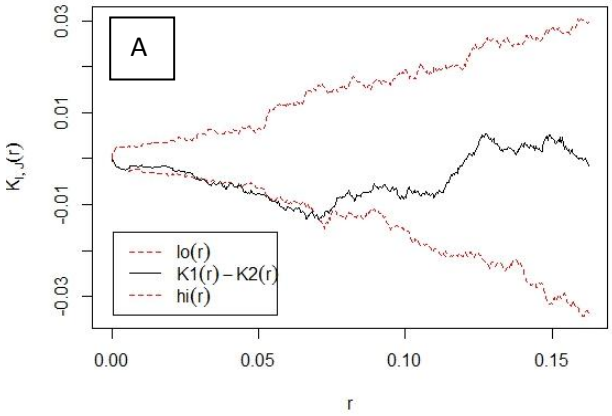
Variable	Mixed % (95%CI) n=274	Cattle % (95%CI) n=292	Pigs only % (95%CI) n=172	Total % (95%CI) n=738	Chi squared
Personal hygiene					
Wear protective clothing	69(66-73)	49(46-51)	27(23-30)	53(51-55)	X^2=79.82, df=2 p<0.001
Wear boots	68(64-71)	41(38-44)	22(19-26)	49(46-51)	X^2=95.14, df=2 p<0.001
Soap available	50(46-54)	62(59-65)	68(64-72)	58(56-60)	X^2=16.40, df=2 p<0.001
Eat at the slaughterhouse	27(23-30)	5(5-7)	37(33-41)	21(20-23)	X^2=70.06, df=2 p<0.001
Smoke daily	22(19-25)	27(24-29)	19(16-22)	23(21-25)	X^2=2.88, df=2 p=0.010
Take alcohol daily	33(30-37)	31(28-33)	32(28-36)	32(30-34)	X^2=2.13, df=2 p=0.032
Drunk at interview	12(9-14)	13(11-15)	5(4-7)	11(10-12)	X^2=6.66, df=2 p<0.001
Use the latrine everytime	73(70-76)	78(75-80)	78(75-82)	76(74-78)	X^2=2.44, df=2 p=0.024
Meat inspection					
Meat inspector visits	98(97-99)	99(99-100)	84(81-87)	96(95-96)	X^2=59.91, df=2 p<0.001
Antemortem exam	44(41-48)	48(45-51)	34(30-38)	44(42-46)	X^2=12.99, df=2 p=<0.001
Slaughter sick animal	23(19-26)	14(12-15)	15(12-18)	18(16-19)	X^2=9.12, df=2 p<0.001
Zoonoses awareness					
Know animals give disease to people	34(31-38)	30(27-32)	29(25-33)	31(29-33)	X^2=2.47, df=2 p=0.020
Know disease can be transmitted from meat	45(41-49)	38(35-40)	42(38-46)	42(40-44)	X^2=2.96, df=2 p=0.009
Named a zoonosis	8(6-10)	8(6-10)	9(6-11)	8(7-9)	X^2=0.11, df=2 p=0.859
Named a disease from meat	9(6-11)	8(6-10)	7(5-9)	8(7-9)	X^2=0.49, df=2 p=0.492

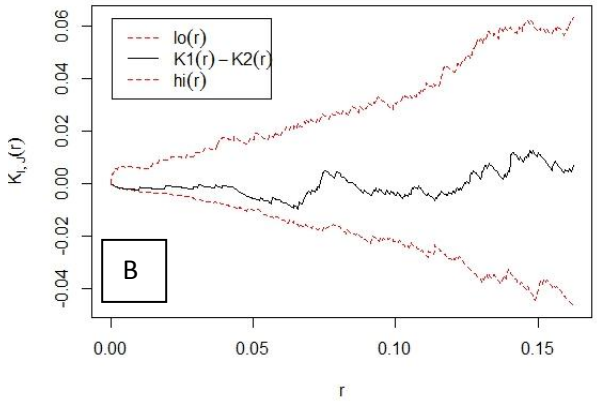
Table A6: Health status of the slaughterhouse workers in western Kenya at the time of interview.

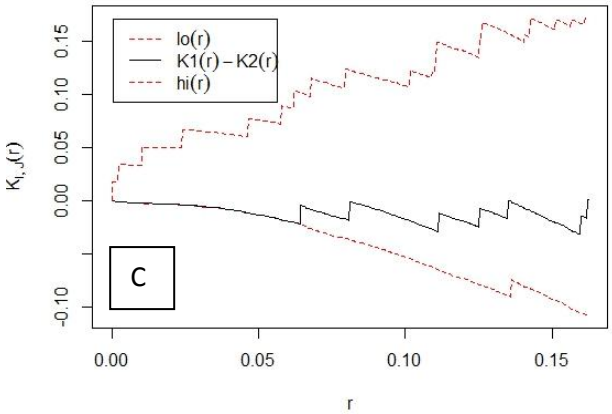
Variable	**Mixed %(95%CI) n=274**	**Cattle % (95%CI) n=292**	**Pigs only % (95%CI) n=172**	**Total % (95%CI) n=738**	**Chi squared**
Recent illness (visit to doctor in past 3 months)	18(15-21)	18(16-20)	16(13-19)	18(16-19)	X^2=0.30, df=2 p=0.613
Previously had Tuberculosis	7(5-9)	1(1-2)	1(0-1)	4(3-4)	X^2=17.39, df=2 p<0.001
Previously had Brucellosis	2(1-3)	1(0-1)	1(0-1)	1(1-2)	X^2=1.63, df=2 p=0.048
Malaria (post 12 months)	50(46-54)	45(42-47)	46(42-50)	47(45-49)	X^2=1.67, df=2 p=0.067
Typhoid (past 12 months)	17(14-20)	11(10-13)	8(6-10)	13(12-14)	X^2=8.32, df=2 p<0.001
Respiratory illness (past 12 months)	14(11-17)	9(8-11)	5(3-7)	10(9-12)	X^2=10.11, df=2 p<0.001
Gastrointestinal illness (last 12 months)	6(4-7)	3(2-4)	1(0-2)	4(3-4)	X^2=6.18, df=2 p<0.001
Sustain injuries at work at least once a month	25(22-29)	25(22-27)	26(22-29)	25(23-27)	X^2=0.05, df=2 p=0.927
Have wounds at the time of examination	7(5-9)	9(8-11)	8(6-10)	8(7-9)	X^2=1.26, df=2 p=0.150
Antibiotic (past 3 months)	19(16-22)	17(15-19)	14(11-17)	17(16-19)	X^2=1.55, df=2 p=0.08
Anti-inflammatory (past 3 months)	44 (40-48)	43(41-46)	52(47-56)	45(43-47)	X^2=3.13, df=2 p=0.007
Antimalarial (past 3 months)	20(17-23)	25(23-28)	23(20-27)	23(21-24)	X^2=2.34, df=2 p=0.022

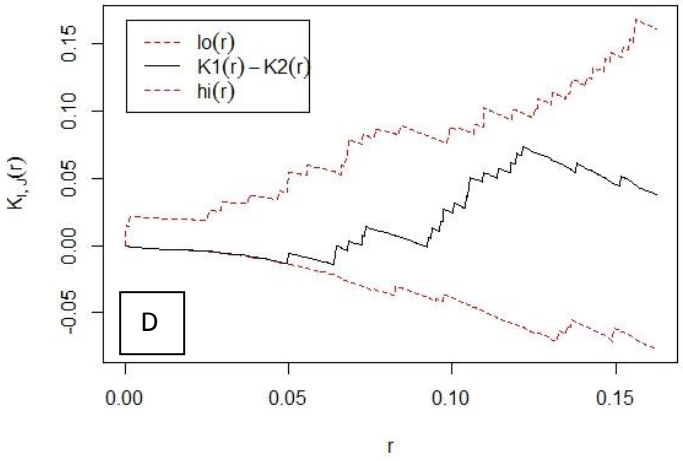
Appendix 6 Spatial analysis

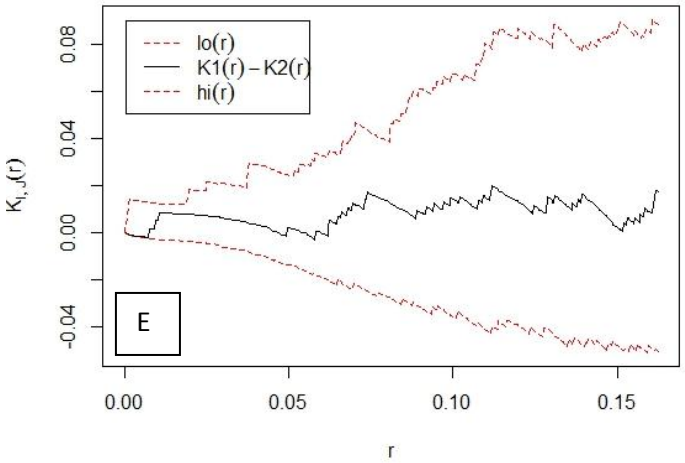
Figure A1 Differences between univariable K functions to test for spatial clustering for 5 zoonoses in slaughterhouse workers in western Kenya, 2012. The difference between the univariable K functions is denoted here by K1-K2 while r represents the radius. Upper and lower 95%confidence limits for the K functions are indicated by dotted lines. A) Leptospirosis, B) Q fever, C) RVF, D) Taeniasis, E) Cysticercosis








lo(r)
K1(r) – K2(r)
hi(r)
D
0.00
0.05
0.10
0.15
-0.05
0.00
0.05
0.10
0.15
r
K1, J(r)


lo(r)
K1(r) – K2(r)
hi(r)
E
0.00
0.05
0.10
0.15
-0.04
0.00
0.04
0.08
r
K1, J(r)

Appendix 7 Images for zoonotic disease recognition

Anthrax

http://commons.wikimedia.org/wiki/File:Anthrax_PHIL_2033.png

https://people.uwec.edu/piercech/bio/Pictures/cutaneous%20anthrax.jpg

Bovine tuberculosis

http://www.fao.org/docrep/003/t0756e/T0756E68.jpg

http://www.michigan.gov/images/deerlungsnodules1_74474_7.jpg

Brucellosis

http://www.vetnext.com/fotos/BRU_006.jpg

http://www.fao.org/docrep/003/t0756e/T0756E71.jpg

Cysticercosis

http://smallfarms.oregonstate.edu/sites/default/files/sfnarchive_img/sp12cysticercosis.jpg

Echinococcosis

http://www.michigan.gov/images/echinococcusgranulosis2_124096_7.jpg

Appendix 8 Variables for multivariable models Chapter 5

Table A7 Full list of slaughterhouse level variables used for multivariable analysis of slaughterhouse worker seropositivity

Variable
Structural
Roof present
Cement floor
Solid walls
Electricity
Sanitation
Toilet
Piped water
Source of water Borehole Municipal River Well
Hand-washing place
Cleaned with soap
Dogs present
Rats present
Personal hygiene
Protective clothing worn
Worker buys clothing
Boots worn
Worker buys boots
Soap provided
Meat inspection
Meat inspector visits daily
Antemortem exam
Slaughter a sick animal

Table A8 Full list of individual level variables used for multivariable analysis of slaughterhouse worker seropositivity

Variable	**Personal hygiene**
Gender	Wear protective clothing
Male	Wear boots
Female	Soap available
Age groups (years)	Eat at the slaughterhouse
18-27	Smoke daily
28-37	Take alcohol daily
38-47	Drunk at interview
49+	Use the latrine everytime
Education	Sustain injuries at work at least once a month
None	**Meat inspection**
Primary	Meat inspector visits
Secondary	Antemortem exam
Duration of work (years)	Slaughter sick animal
<5	**Zoonoses awareness**
6-10	Know animals give disease to people
11-15	Know disease can be transmitted from meat
16+	Named a zoonosis
Job in the slaughterhouse	Named a disease from meat
Cleaner	**Health**
Cleans intestines	HIV
Flayer	Wounds at time of interview
Slaughterman	Febrile at interview
Other	Malaria diagnosed
Water source	*Entamoeba histolytica*
Borehole	*Iodamoeba butschulii*
Spring	*Ascaris* spp
River	Hookworm
Well	*Trichuris* spp.
Municipal	*S. mansoni*

Appendix 9 PAZ individual questionnaire

1 Start date and time
Please tick the box to automatically record the start time
2 Recorder ID
1-Eric; 2-Lian; 3-James; 4-Omoto; 5-Fredrick; 6-Jenipher
3 Homestead ID [barcode]
Please can the barcode
4 Does that participant meet the selection criteria (age over 5, not in last trimester of pregnancy)?
Has informed consent been acquired?
5 Interviewee ID [barcode]
Please STICK the barcode in the file and SCAN it here
6 Language of questionnaireadministration
Teso, Samia, Bukusu, Luhya, Luo, Swahili
7 Participants age
NR, <1, 1-4, 5-9, 10-14, 15-19, 20-24, 25-29, 30-34, 35-39, 40-44, 45-49, 50-54, 55-59, 60-64, 65-69, 70-74, 75-79, 80+
8 Participants sex
Male, Female, ND
9 Tribal origin
Teso, Luhya, Luo, Kikuyu, Samia, Saboat
10 Prinicipal religion (or the individual themselves)
Roman catholic, Protestant, Pentacostal, Baptist, Other Christian, Muslim
11Marital status
Single, Married, Divorced, Widowed, NR
12 How long have you lived in this village?
<1yr, 1-5 years, >5 years, NR
13 How many pregnancies have you had?
NR, None, 1, 2, 3, 4
14 Are you pregnant at present?
Yes, No, NR
15 If pregnant, what stage of pregnancy?
1st trimester, 2nd trimester, 3rd trimester
16 How many living children do you have?
NR, 1, 2, 3, 4, 5
17 What level of education have you reached?
No formal education, Pre-school, Primary, Secondary, Tertiary, College
18 What is your major occupation? Select ONE
Farmer, Trader, Shop keeper, Full time parent, Student, Driver
19 If other, please enter details of occupation
20 How many days do you leave your village each week?
Eg how many days did you leave the village THIS week?
Never, Less than once per week, 1, 2, 3. 4
21 On average, how many hours do you spend outside the village on each trip?
22 Do you have contact with dogs?
Never, Daily, At least once per week, At least once a month, At least once a year, Used to but not anymore
23 Is there a dog living on this compound?
Yes, No, Don't know, ND

24 How long has any dog lived here?
<1yr, 1-3 years, >3yrs, Unknown
25 Do you feed internal organs of animals to this dog?
Yes, No, Don't know, ND
26 Does/do your dog(s) ever receive drugs or injections?
Yes, No, Don't know, ND
27 What drugs/vaccines did the dog receive?
Worms, Mange, Fleas, Ticks, Vomiting, Diarrhoea
28 Is there a cat on the compound?
Yes, No, Don't know, ND
29 Do you have contact with any cats? (even if one dosen't live on this compound)
Never, Daily, At least once per week, At least once a month, At least once a year, Used to but not anymore
30 In the last 12 months, have you been hunting?
No, Daily, At least once per week, At least once a month, At least once a year, Used to but not anymore
31 In the last 12 months, have you been fishing (river or lake)?
No, Daily, At least once per week, At least once a month, At least once a year, Used to but not anymore
32 In the last 12 months, have you been involved with taking animals (your own or someone elses) for grazing?
No, Daily, At least once per week, At least once a month, At least once a year, Used to but not anymore
33 In the last 12 months, have you been involved with feeding livestock within or outside the home?
No, Daily, At least once per week, At least once a month, At least once a year, Used to but not anymore
34 In the last 12 months, have you been involved with milking cattle within or outside the home?
No, Daily, At least once per week, At least once a month, At least once a year, Used to but not anymore
35 In the last 12 months, have you been invovled in milking goats or sheep within or outside the home?
No, Daily, At least once per week, At least once a month, At least once a year, Used to but not anymore
36 In the last 12 months, have you been involved with dealing with births of new animals within or outside the home?
No, Daily, At least once per week, At least once a month, At least once a year, Used to but not anymore
37 In the last 12 months, have you been involved with handling animals that have aborted or aborted material?
No, Daily, At least once per week, At least once a month, At least once a year, Used to but not anymore
38 In the last 12 months have you been invovled with the slaughter of animals within or outside the home?
No, Daily, At least once per week, At least once a month, At least once a year, Used to but not anymore
39 In the last 12 months, have you been involved with manure preparation within or outside the home?

No, Daily, At least once per week, At least once a month, At least once a year, Used to but not anymore
40 In the last 12 months, have you been involved with skinning dead animals within or outside the home?
No, Daily, At least once per week, At least once a month, At least once a year, Used to but not anymore
41 In the last 12 months, have you been involved with burying dead animals within or outside the home?
No, Daily, At least once per week, At least once a month, At least once a year, Used to but not anymore
42 Do livestock have access to the building you sleep in?
Yes, No, NR
43 Which livestock have access to the buildings in which you sleep?
(Tick all that apply)
Cattle, Pigs, Goats, Sheep, Chickens, Ducks
44 In the last 12 months, have you seen wildlife around the home?
No, Daily, At least once per week, At least once a month, At least once a year, NR
45 What wildlife have you seen around the home? (any non domestic species should be listed; tick all that apply).
Bushbuck, Other bovid, Bats, Mongoose, Snake, Monitor lizard
46 In the last 12 months, have you seen rats around the home?
Daily, Sometimes, Never, NR
47 Food preferences section
48 Do you ever eat meat?
Yes, No, NR
49 How often do you eat beef?
Never, Daily, At least once a week, At least once per month, At least once per year, Only on special occasions
50 How do you like your beef cooked?
Boiling, Barbeque, Fried, Dried, Smoked, Raw
51 To what extent do you like your beef cooked?
Still red, Slightly pink, Brown on outside, Brown all through, Raw, Other
52 How often do you eat pork?
Never, Daily, At least once a week, At least once per month, At least once per year, Only on special occasions
53 How do you like your pork cooked?
Boiling, Barbeque, Fried, Dried, Smoked, Raw
54 To what extent do you like your pork cooked?
Juicy, Dry, White, With blood, Fully roasted, Still red
55 Would you consider buying pork that looks like this
(SHOW PICTURE OF PORK WITH CYSTS)
Frequently, Sometimes, Never, NR
56 In the last 12 months, where have you obtained meat? Tick all that apply.
I have never obtained meat, Butchery shop, Market, Own animals, Neighbour, Family member
57 In the last 12 months, have you eaten meat outside the home?
Yes, No, NR
58 In the last 12 months, where have you eaten meat outside the homestead? Tick all that apply.
Neighbour, Roadside, Hotel, School, Other

59 In the last 12 months, have you drunk cows milk?
Yes, No, NR
60 How often do you drink cows milk?
Daily, At least once a week, At least once per month, At least once per year, Only on special occasions, NR
61 Where do you obtain cows milk?
Own herd, Neighbour, Shop, Market, Other, NR
62 In the last 12 months, how have you taken cows milk for drinking?
Boiled, Soured, Raw, Pasteurised, Other
63 In the last 12 months, have you drunk goats milk?
Yes, No, NR
64 How often do you drink goats milk?
Daily, At least once a week, At least once per month, At least once per year, Only on special occasions, NR
65 Where do you obtain goats milk?
Own herd, Neighbour, Shop, Market, Other, NR
66 In the last 12 months, how have you taken goats milk?
Boiled, Soured, Raw, Pasteurised, Other
67 In the last 12 months, have you drunk animal blood?
Yes, No, NR
68 In the last 12 months, how often have you taken animal blood?
Daily, At least once a week, At least once per month, At least once per year, Only on special occasions, NR
69 In the last 12 months, where have you obtained animal blood?
Own herd, Neighbour, Shop, Butchery/slab, Market, Other
70 Water and hygiene section
71 Where did you obtain your water from in the last wet season?
Borehole, River, Pump, Tap, Well, Spring
72 Where did you obtain your water from in the last dry season?
Borehole, River, Pump, Tap, Well, Spring
73 Are you involved in collecting water?
Yes, No, NR
74 This month, have you treated water before drinking it?
No, Boil, Add chlorine, Add iodine, Filter, Other
75 In the last month, how often have you used the latrine when you need to defecate?
Always, Frequently, Sometimes, Never, NR
76 Health status section
77 Have you had a period of illness in the past 12 months?
Yes, No, NR
78 If you have had a health problem, please list
79 Usually when you feel ill, where do you seek treatment?
Don't seek treatment, Family member, Community health worker, Traditional healer, Chemist, Private clinic
80 Have you ever had worms in your faeces?
Yes, No, Don't know, NR
81 If you have had worms in your faeces, when was the last episode?
Currently ill, <1week ago, <1 month ago, 1-3 months ago, 3-6 months ago, 6 mon-1yr ago
82 Where did you seek treatment for worms?
Don't seek treatment, Family member, Community health worker, Traditional healer, Chemist, Private clinic

83 Have you ever seen blood in your urine?
Yes, No, Don't know, NR
84 If you have had blood in your urine, when was the last episode?
Currently ill, <1week ago, <1 month ago, 1-3 months ago, 3-6 months ago, 6 mon-1yr ago
85 Have you ever had problems breathing?
Yes, No, Don't know, NR
86 If you have had problems breathing, when was the last episode?
Currently ill, <1week ago, <1 month ago, 1-3 months ago, 3-6 months ago, 6 mon-1yr ago
87 Describe symptoms of breathing problems
88 Where did you seek treatment for breathing difficulties?
Don't seek treatment, Family member, Community health worker, Traditional healer, Chemist, Private clinic
89 Have you ever had fever?
Yes, No, Don't know, NR
90 If you have had fever, when was the last episode?
Currently ill, <1week ago, <1 month ago, 1-3 months ago, 3-6 months ago, 6 mon-1yr ago
91 Where did you seek treatment for this fever?
Don't seek treatment, Family member, Community health worker, Traditional healer, Chemist, Private clinic
92 Have you had diagnosed malaria?
Yes, No, Don't know, NR
93 If yes, when was the last episode?
Currently ill, <1week ago, <1 month ago, 1-3 months ago, 3-6 months ago, 6 mon-1yr ago
94 If you have had diagnosed malaria, where did you seek treatment?
Don't seek treatment, Family member, Community health worker, Traditional healer, Chemist, Private clinic
95 Have you ever had a dog bite?
Yes, No, Don't know, NR
96 If you have had a dog bite, when did this last occur?
<1week ago, <1 month ago, 1-3 months ago, 3-6 months ago, 6 mon-1yr ago, >1yr ago
97 In response to this dog bite, what did you do?
Nothing, Received injection, Received several injections, Obtained tablets, Consulted health worker, Taken to hospital
98 If other treatment for dog bite, enter details
99 Have you been bitten by another animal in the last 12 months?
No, Cat, Rat, Pig, Cow, Goat/sheep
100 When was this other animal bite?
<1week ago, <1 month ago, 1-3 months ago, 3-6 months ago, 6 mon-1yr ago, >1yr ago
101 Are you aware of ever having had brucellosis?
Yes, No, Don't know, NR
102 Are you aware of ever having had tuberculosis?
Yes, No, Don't know, NR
103 Are you aware of ever having had q-fever?
Yes, No, Don't know, NR
104 Are you aware of ever having had sleeping sickness?
Yes, No, Don't know, NR
105 Are you aware of ever having had tapeworm?
Yes, No, Don't know, NR
106 Are you aware of ever havinghad Rift Valley Fever?
Yes, No, Don't know, NR

107 Have you taken any medicines in the last one month?
Yes, No, Don't know, NR
108 If you have taken medicines in the last month, tick all that apply...
Unknown, Chloroquine, Other antemalarial, Anti-retroviral, Antibiotic, Anti-tussives
109 Are you currently taking any medicines?
Yes, No, Don't know, NR
110 If you are currently taking medicines, please enter all that apply...
Unknown, Chloroquine, Other antemalarial, Anti-retroviral, Antibiotic, Anti-tussives
111 Have you had the following in the last 3 months: fever/chills?
Yes, No, Don't know, ND
112 Have you had the following in the last 3 months: joint pain?
Yes, No, Don't know, ND
113 Have you had the following in the last 3 months: backache?
Yes, No, Don't know, ND
114 Have you had the following in the last 3 months: headache?
Yes, No, Don't know, ND
115 Have you had the following in the last 3 months: loss of vision?
Yes, No, Don't know, ND
116 Have you had the following in the last 3 months: chest pain?
Yes, No, Don't know, ND
117 Have you had the following in the last 3 months: general weakness?
Yes, No, Don't know, ND
118 Have you had the following in the last 3 months: lack of coordination?
Yes, No, Don't know, ND
119 Have you had the following in the last 3 months: seizures?
Yes, No, Don't know, ND
120 Have you had the following in the last 3 months: confusion?
Yes, No, Don't know, ND
121 Have you had the following in the last 3 months: loss of appetite?
Yes, No, Don't know, ND
122 Have you had the following in the last 3 months: sudden weight loss?
Yes, No, Don't know, ND
123 Have you had the following in the last 3 months: cough?
Yes, No, Don't know, ND
124 Have you had the following in the last 3 months: shortness of breath?
Yes, No, Don't know, ND
125 Have you had the following in the last 3 months: adenitis?
Yes, No, Don't know, ND
126 Have you had the following in the last 3 months: abdominal discomfort?
Yes, No, Don't know, ND
127 Have you had the following in the last 3 months: nausea/vomiting?
Yes, No, Don't know, ND
128 Have you had the following in the last 3 months: diarrhoea?
Yes, No, Don't know, ND
129 Have you attended hospital in the last 5 years?
Yes, No, Don't know, ND
130 What was the visit to hospital for?
Injury, Childbirth, Acute sickness, Fever or malaria, TB, Sleeping sickness
131 If visited hospital for another reason, please enter details
132 Do you smoke cigarettes?

Daily, Weekly, Monthly, On special occasions, Previously but not anymore, Never
133 If daily or weekly, what is the number of cigarettes you smoke per week?
134 Do you know if you were vaccinated as a child?
Yes, No, Don't know, NR
135 Do you know if you have ever received vaccinations against the following disease: rabies?
Yes, No, Don't know, NR
136 Do you know if you have ever received vaccinations against the following disease: TB?
Yes, No, Don't know, NR
137 Do you know if you have ever received vaccinations against the following disease: tetanus?
Yes, No, Don't know, NR
138 Do you know if you have ever received vaccinations against the following disease: polio?
Yes, No, Don't know, NR
139 Do you know if you have ever received vaccinations against the following disease: influenza?
Yes, No, Don't know, NR
140 Do you know if you have ever received vaccinations against the following disease: mumps?
Yes, No, Don't know, NR
141 Do you know if you have ever received vaccinations against the following disease: measles?
Yes, No, Don't know, NR
142 Do you know if you have ever received vaccinations against
the following disease: rubella?
Yes, No, Don't know, NR
143 Do you know if you have ever received vaccinations againstthe following disease: typhoid?
Yes, No, Don't know, NR
144 Do you know if you have ever received vaccinations against the following disease: diptheria?
Yes, No, Don't know, NR
145 Do you know if you have ever received vaccinations against the following disease: cholera?
Yes, No, Don't know, NR
146 Do you know if you have ever received vaccinations against the following disease: hepatitis A?
Yes, No, Don't know, NR
147 Do you know if you have ever received vaccinations against the following disease: hepatitis B?
Yes, No, Don't know, NR
148 Do you know if you have ever received vaccinations againstthe following disease: meningitis?
Yes, No, Don't know, NR
149 Do you know if you have ever received vaccinations againstthe following disease: yellow fever?
Yes, No, Don't know, NR
150 Knowledge about food-borne and zoonotic diseases
151 Are you aware of any diseases you might catch from contact with animals?

Yes, No, Don't know, NR
152 Which symptoms/diseases might you catch from cattle?
Unknown diseases, Malaria, Fever, Stomach pain, Diarrhoea, Respiratory difficulties
153 Which diseases/symptoms might you catch from pigs?
Unknown diseases, Malaria, Fever, Stomach pain, Diarrhoea, Respiratory difficulties
154 Which diseases/symptoms might you catch from sheep or goats?
Unknown diseases, Malaria, Fever, Stomach pain, Diarrhoea, Respiratory difficulties
155 Which symtoms/diseases might you catch from dogs or cats?
Unknown diseases, Malaria, Fever, Stomach pain, Diarrhoea, Respiratory difficulties
156 Are you aware of any diseases you might catch from eating meat?
No, Don't know, Unknown diseases, Malaria, Stomach pain, Diarrhoea
157 Direct measurements section
158 Weight (kg)
159 Height (cm)
160 Mid-Upper Arm Circumference (MUAC) (mm) If over 340mm enter 341
161 Temperature (degrees C)
162 Splenomegaly
Yes, No, Don't know, Refused Examination
163 Hepatomegaly
Yes, No, Don't know, Refused Examination
164 Abdominal distension
Yes, No, Don't know, Refused Examination
165 Membrane palour
Yes, No, Don't know, Refused Examination
166 Jaundice
Yes, No, Don't know, Refused Examination
167 Limb oedema
Yes, No, Don't know, Refused Examination
168 Rash
Yes, No, Don't know, Refused Examination
169 Wounds requiring treament?
Yes, No, Don't know, Refused Examination
170 BARCODE: Take a red top blood sample. Please scan the barcode
171 BARCODE: Take a green top blood sample. Please scan the barcode
172 BARCODE: Take a purple top blood sample. Please scan the barcode
173 Was a stool sample collectedfrom this participant?
Yes, No
174 BARCODE: Scan the stool sample barcode for thisparticipant.
175 BARCODE: Please scan the barcode for the thick film
176 BARCODE: Please scan the barcode for the thin film
177 Almost finished! Tick the box to enter the time and date of questionnaire completion.

Appendix 10 PAZ homestead questionnaire

1 Enter date and time start
2 Homestead unique ID [BARCODE]
3 Name of household head
4 Sex of the respondent
Male, Female, ND
5 Language of questionnaire administration
Teso, Samia, Bukusu, Luhya, Luo, Swahili
6 If the answer was "Other language," please type the language name here
7 How many people currently live in the homestead? (by homestead we mean all dwellings within the compound)
8 How many people
9 Field: How many people aged 1-4
10 How many people aged 5-9
11 How many people aged 10-14
12 How many people aged 15-19
13 How many people aged 20-24
14 How many people aged 25-29
15 How many people aged 30-34
16 How many people aged 35-39
17 How many people aged 40-44
18 How many people aged 45-49
19 How many people aged 50-54
20 How many people aged 55-59
21 How many people aged 60-64
22 How many people aged 65-69
23 How many people aged 70-74
24 How many people aged 75-79
25 How many people aged 80-84
26 How many people 85+
27 autocount age
28 Age sum checker
If the ages do not add up, you will see an error. If no error appears, click NEXT and proceed.
29 Livestock ownership census Please now enter details relating to livestock ownership
30 Does this homestead keep cattle?
Yes, No, ND
31 Cattle: number of male calves
32 Cattle: number of female calves
33 Cattle: number of weaned males
34 Cattle: number of weaned females
35 Cattle: number of adult male (castrate)
36 Cattle: number of adult males (entire)
37 Cattle: number of adult females
38 Does this homestead keep pigs?
Yes, No, ND
39 Pigs: number of suckling piglets
40 Pigs: weaned males not yet mated
41 Pigs: weaned females non parous

42 Pigs: sows pregnant or parity
43 Pigs: boars that have bred
44 Does this home keep sheep?
Yes, No, ND
45 How many sheep does this home keep?
46 Does this home keep goats?
Yes, No, ND
47 How many goats does this homestead keep?
48 Does this home keep chickens?
Yes, No, ND
49 Does this home keep ducks?
Yes, No, ND
50 Does this home own/keep dogs?
Yes, No, ND
51 Does this home own/keep cats?
Yes, No, ND
52 Does this home keep any other livestock?
Yes, No, ND
53 Please enter other livestock kept
54 What is your source of water for cooking/drinking in the DRY season? Tick ALL that apply
Borehole, Dam/Pond, River, Well, Spring, Piped
55 What is your source of water for cooking/drinking in the WET season? Tick ALL that apply
Borehole, Dam/Pond, River, Well, Spring, Piped
56 In the last month, have you treated your water at your homestead before drinking it?
No, Boil, Add chlorine, Add iodine, Waterguard, Aquatabs
57 What is the main cooking fuel in the household?
Open fire – firewood, Open fire – charcoal, Gas stove, Jico stove, Paraffin stove, Solar stove
58 Has your village experienced flooding in last 12 months - to extent that crops were damaged?
Yes, No, ND
59 When was flooding last experienced?
This week, This month, Last 6 months, Last 12 months
60 Has your village experienced drought in the last 12 months, to the extent that plants started to fail?
Yes, No, ND
61 When was drought last experienced?
This week, This month, Last 6 months, Last 12 months
62 Do you grow crops?
Yes, No, ND
63 Why do you grow crops? Tick all that apply
For the homestead, To sell
64 Access to medical care questions Please answer questions relating to medical care
65 Where does the majority of your household access medical facilities?
Community heath workers, Traditional healer, Chemist, Hospital, Health centre, Church healer
66 Medical care access – other, please write response below
67 Distance to most used medical facility (km)
68 How do you normally get to the medical facility?
Walk, Boda boda (bicyle), Boda boda (motorbike),Own bike, Own Motorbike, Matatu
69 How much would it normally cost you to get to the medical facility?

Enter value in KSh
70 Does this homestead keep ANY animals? (you will have already asked this question)
Yes, No
71 Where do you access veterinary services? Tick all that apply
Never accessed, Gov't vet, Private vet, Family/myself, Vet drug supplier, Extension service
72 Where do you access veterinary services - other? If OTHER, enter details
73 Have you used veterinary services in the last 12 months?
Yes, No, ND
74 Does this homestead keep Cattle? (you will already have asked this question)
Yes, No
75 Why do you keep cattle? Tick all that apply
ND, milk for the home, milk to sell, meat for the home, meat to sell, manure
76 Do you ever buy cattle or have you received cattle as a gift from outside the homestead?
Yes, No, ND
77 How long ago did you last buy/acquire new cattle?
<1 month ago, 1<2 months, 2<3 months, 3<6 months, 6months -1 year, >1yr ago
78 Where do you usually buy cattle/receive cattle from?
Market within sublocation, Market in other sublocation, Other source within sublocation, Other source in other sublocation
79 Name of market
80 If bulls are kept do you rent or lend them for breeding
Do you rent your bulls for breeding?
Yes, No, NR
81 Do your cows/bulls engage in communal breeding?
Yes, No, ND
82 Have you ever experienced abortion in your herd?
Yes, No, NR
83 How long ago was the last abortion?
1 month ago, 2 months ago, 3 months ago, Up to 6 months ago, Up to 1yr ago, More than 1yr
84 What did you do with the aborted material?
Left it alone, Buried it, Burnt it, Fed it to animals, Ate it, Took it to the bush
85 How often are your cows milked?
No lactating cattle, Do not milk cows, 1x daily, 2x daily, 3x daily, Once every two days
86 Are cattle herded with goats or sheep?
(the farmers own or other small ruminants)
Yes, No, ND
87 How do you graze/feed cattle in the dry season?
Zero grazing, Herded as single herd, Herded with other herds, Tethered, Free (communal) grazing, NR
88 How do you graze/feed cattle in the wet season?
Zero grazing, Herded as single herd, Herded with other herds, Tethered, Free (communal) grazing, NR
89 Do you use anything to control worms in your cattle?
Yes, No, ND
90 What do you use to control worms in cattle?
Drench (unknown drug), tablets/bolus (unkown drug), Pour on (unknown drug), Injection (unknown drug), Traditional remedy, Albendazole
91 How often do you use this cattle deworming product?

At least once a week, at least once a monthly, Every 2 months, Every 3 months, Every 4 months, Every 5 months
92 Do you control ticks in your cattle?
Yes, No, ND
93 What do you use to control cattle ticks?
Tick grease, Deltamethrin, Amitraz, Paraffin, Used motor oil, Drench - unknown drug
94 How often do you use this cattle tick control product?
at least once a week, at least once a month, Every 2 months, Every 3 months, Every 6 months, When ticks seen
95 Do you control trypanosomes in your cattle?
Yes, No, NR
96 How do you control trypanosomes in cattle?
Spray - unknown drug, Dip - unknown drug, Pour on - unknown drug, Injection - unknown drug, Tsetse traps, Diminazine
97 What other method do you use to control Trypanosomes in your cattle?
98 How often do you use any of these trypanosome control products?
at least once a week, at least once a month, Every 2 months, Every 3 months, Every 4 months, Every 5 months
99 Do you control Coccidia in your cattle?
Yes, No, NR
100 Which drug do you use to control Coccidia in cattle?
Imidocarb, Parvaquone, Buparvaquone, Toltrazuil, Injection - unknown drug, Drench - unknown drug
101 How often do you use this Coccidia control drug?
Weekly, Monthly, Every 1 month, Every 2 months, Every 3 months, Every 4 months
102 Where do you purchase these drugs for your cattle?
Government vet, Private vet, Agrovet, Chemist, Neighbour, Home made
103Other source of cattle drugs not listed above? If No, click NEXT...
104 Have any cattle in the home been given any vaccinations?
No, Unknown vaccine, ECF, BRSV, CBPP, PI-3
105 What is the water source for your cattle in the dry season (tick ALL that apply)
Borehole, Dam/pond/other standing water, Animals go to river, Collected from river, Covered well, Open well
106 What is the source of water for your cattle in the wet season (tick ALL that apply)
Borehole, Dam/pond/other standing water, Animals go to river, Collected from river, Covered well, Open well
107 Does the home keep pigs (you may have already answered this question)
Yes, No, ND
108 Why do you keep pigs (tick ALL that apply)
Home consumption, To sell piglets, To sell for meat, To give as gifts, Dowry, As pet
109 How do you house pigs in the dry season (tick ALL that apply)
Free roaming, Tethered, In stall, In kraal, In yard
110 How do you feed your pigs in the dry season? (tick ALL that apply)
Crops grown for pigs, Waste from house, Waste from neighbours, Waste from commercial places, Commercial feed, tethered no supplement
111 How do you house pigs in the wet season?
Free roaming, Tethered, In stall, In kraal, In yard
112 How do you feed your pigs in the wet season? (Tick ALL that apply)
Crops grown for pigs, Waste from house, Waste from neighbours, Waste from commercial places, Commercial feed, tethered no supplement

113 Please repeat: are pigs fed waste (from homestead or hotels) during any season?
Yes, No, ND
114 If pigs are fed waste, do you cook it prior to feeding to the pigs?
Yes, No, ND
115 Please repeat: are pigs housed during any season?
Yes, No, ND
116 What is the flooring in the pig housing?
Mud, Concrete, Slatted, Other
117 Do you use anything to control worms in your pigs?
Yes, No, ND
118 What do you administer to control worms in pigs?
Don't know, Drench (unknown drug), Tablet/bolus (unknown drug), Pour on (unknown drug), Injection (unknown drug), Albendazole
119 How often do you use this pig worm control product?
at least once a week, at least once a month, Every 2 months, Every 3 months, Every 4 months, Every 5 months
120 Do you control ticks in your pigs?
Yes, No, ND
121 What do you use to control ticks in pigs?
Tick grease, Deltamethrin, Amitraz, Paraffin, Used motor oil, Drench - unknown drug
122 How often do you use this pig tick control product?
at least once a week, at least once a month, Every 2 months, Every 3 months, Every 4 months, Every 5 months
123 Where do you purchase these drugs for your pigs?
Government vet, Private vet, Agrovet, Chemist, Neighbour, Home made
124 Have the pigs on the homestead been vaccinated against anything?
Yes, No, Don't know, ND
125 Pig vaccine - does the farmer know which vaccine it was?
Enter YES for known vaccine
Enter NO for unknown vaccine
Yes, No, ND
126 Name of pig vaccine if known
127 Are there any significant problems with your pigs?
No, Worms, Ticks, ASF, Sudden mortality, unknown cause, Dystocia
128 Some questions about the slaughtering of animals will follow
129 Do you ever slaughter your own animals?
Yes, Only Chickens, No, NR
130 Where do you slaughter cattle?
At the homestead, Away from homestead, Don't slaughter cattle, NR
131 How often do you slaughter cattle?
Daily, Weekly, Monthly, Special occasions only, At least once every 6 months, At least once per year
132 Where do you slaughter your pigs?
At the homestead, Away from homestead, Don't slaughter pig, NR
133 How often do you slaughter pigs?
Daily, Weekly, Monthly, At least once in 6 months, At least once per year, Less than once per year
134 Do you slaughter goats or sheep at home?
Yes, No, Don't know, NR
135 Are your pigs inspected at slaughter?

Yes, No, Don't know, NR
136 Who inspected the meat for cysts?
Myself, Neighbour, Government inspector, Private vet, Other
137 If cysts are found, what do you do with the meat?
Have never found cysts, Dispose of carcass, Cut out cycts and use meat, Sell to others, Treat as normal, Other
138 Do you buy meat from the butcher/market?
Yes, No, Don't know
139 How many houses (dwellings occupied by humans) are there on the compound?
140 Roof - how many with iron sheets?
141 Roof - how many with thatch?
142 Roof- how many with tiles?
143 Roof: how many with other materials?
144 The result of your data entry for roof questions is:
Field: Roof sum checker If the roof types do not add up, you will see an error. If no error appears, click NEXT and proceed.
146 Walls: how many with mud (no bricks)?
147 Walls: how many with unburnt bricks?
148 Walls: how many burnt mud bricks?
149 Walls: count burnt bricks and cement?
150 Walls: count mud with cement?
Field: Walls: how many with timber walls?
152 Walls: how many cement only?
153 Walls: how many stone walls?
154 Walls: how many with other materials?
155 Walls calc checker
156 Walls sum checker If the wall types do not add up, you will see an error. If no error appears, click NEXT and proceed.
157 Floor: how many have earth?
158 Floors: how many cemented?
159 Floors: how many tiled?
160 Floors: how many wooden?
161 Floor: how many other materials?
162 Floor calc checker
163 Floor sum checker If the floor types do not add up, you will see an error. If no error appears, click NEXT and proceed.
164 Is there a latrine in the compound?
Yes, No, ND
165 What type of latrine is there on the compound? (this question is mainly to determine possible animal access, so completely closed
Latrine completely closed, Partially closed, Open pit
166 Is there evidence of latrine use on a regular basis?
Yes, No, ND
167 Is there evidence of scavenging by animals around the latrine?
Yes, No, ND
168 Electricity: do you have the following in your home? Tick ALL that apply
Electricity (from mains), Electricity from generator, Electricity from a car battery, Electricity from solar power, None of the above
169 Household electric goods: do you have the following in your home? Tick ALL that APPLY

Radio, Mobile phone, Mobile phone charger, Television, None of the above
170 Furniture: do you have the following in your home? Tick ALL that APPLY
Cupboard, Wooden bed, Bednet, Sofa with cushions, Clock, Watch
171 Transport: do you have the following in your home? Tick ALL that APPLY
Bicycle, Motorbike, Car, None of the above
172 Have you ever been involved in any other programmes (research, government, interventions, medical or veterinary?
Yes, No, Don't know
173 If other programmes, please explain
174 This is the end of the questionnaire to the farmer. Please THANK the farmer for their participation
175 GPS northing
176 GPS easting
177 GPS elevation Please enter the altitude as recorded by the GPS (in metres)
178 GPS code Enter the code given to the GPS unit used
179 Enter the record number recorded in the GPS device
180 Enter date and time end

Appendix 11 Variables for multivariable models Chapter 7

Table A9 Full list of individual and homestead level variables used for multivariable analysis of participant seropositivity

Variable	**Risk factors for zoonoses**
Gender	Home slaughter
Male	Skinning animals
Female	Deal with animals births
Age	Handling abortion material
<35	Handling manure
≥35	Milking cows
Education	Milking goats
None	Hunting
Primary	Contact with dogs
Secondary	See rats around homestead
Occupation	Drink animal blood
Farmer	Eat beef
Other	Eat pork
Dietary	Drink cow's milk
Eat beef	Drink goat's milk
Eat pork	Keep cattle
Drink cows milk	Keep sheep
Drink goats milk	Keep goats
Health	Keep pigs
HIV	Keep chickens
Wounds at time of interview	**Miscellaneous**
Febrile at interview	Smoking behaviour
Malaria diagnosed	Latrine use
Entamoeba histolytica	**Water source**
Iodamoeba butschulii	Borehole
Ascaris spp	Spring
Hookworm	River
Trichuris spp.	Well
S. mansoni	Municipal

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